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Title: Field evaluation of three topically applied insect repellent products containing IR3535 against mosquitoes in Florida.

Protocol Version 5
23rd April 2017

Study Center:

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Sponsor and Funder:

LivFul Inc.
Address: 2972 Webb Bridge Rd.
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USA

Trial Monitoring Center:

arctec
Address: Room LG38, LSHTM, Chariot Innovations Ltd., Keppel St, London WC1E 7HT, UK
Tel: +44 (0) 20 7927 2883
Email: arctec@lshtm.ac.uk

This protocol provides information about procedures for entering participants into repellent trials. The protocol should not be used as a guide for the treatment of others; every care was taken in its drafting, but corrections or amendments may be necessary. Problems relating to this trial should be referred, in the first instance, to the Principal Investigator.

This trial will adhere to the principles outlined in the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations.

Commented [AM1]: Recommend adding statement of intention to comply with good laboratory practices in addition to good clinical practices.

Study Synopsis

Name of Sponsor:	LivFul Inc.		
Name(s) of Product:	Three LivFul Inc. mosquito repellent products (Lotion, Spray, Wipe)		
Title of Study:	Field evaluation of three topically applied insect repellent products containing IR3535 against mosquitoes in Florida		
Short Title:	Field repellent testing in Florida		
Protocol Number:	2	Version:	V5.0
Type of Product:	Spray, lotion and wipe	Phase of Development:	IV
Principal Investigator:	Dr. Emma Weeks, University of Florida, Gainesville, FL, USA		
Monitoring Investigator:	Arthropod Control Product Test Centre (<i>arctec</i>), London School of Hygiene & Tropical Medicine, London, UK		
Primary/Secondary Objective(s):	Primary objective: To determine the efficacy and duration of protection of three topically applied insect repellent products at preventing landing by mosquitoes. Efficacy will be determined by calculating the complete protection time (CPT), which is defined as the time between application of the repellent product and the occurrence of the first landing in a 5-minute test, followed by a confirmatory landing within 30 minutes.		
Study Design / Methodology:	A two-site field setting study using healthy volunteers to test three insect repellent product formulations (lotion, spray, and wipe) against mosquitoes.		
Number of Participants:	The sample size calculations (based on 90% power and a 5% significance level) require 10 participants. Based on power analysis, a sample size of 13 test subjects for each site/product combination in this study design would provide sufficient power (>0.90) to obtain a ratio of the lower limit of 95% CI of the estimated median CPT / estimated median CPT is ≥ 0.6, where the ratio of the lower limit of 95% CI of the estimated median CPT/estimated median CPT expresses the precision of estimated median CPT. Two additional subjects will serve as untreated controls for each field test to Two untreated participants will also monitor the landing rate throughout the study. An additional 6-5 participants could be will be enrolled as alternates to replace any test subjects who drop out before testing beginsives. In total, up to 20 people could be necessary for each test day. Assuming no subjects participate as test, untreated control, or alternate subjects more than		

Commented [AM2]: Include more specific list of products with % AI, e.g., lotion with 15% IR3535, spray with 17% IR3535, and wipe with 20% IR3535

Commented [AM3]: Include more specific list of products with % AI, e.g., lotion with 15% IR3535, spray with 17% IR3535, and wipe with 20% IR3535

	<u>once, total of 120 people could be necessary to complete all testing outlined in this protocol. alternatives.</u>
Intended Product Users:	The test product is designed for use by the general public in areas where mosquito biting is likely.
Study Duration:	<p>Participants will undergo a screening evaluation which includes <u>the consent process</u>, a training (to detect mosquito landings and use an aspirator), <u>and a mosquito attraction test</u>, and a dose determination assay. <u>Subjects recruited into the study will</u> Followed by participation <u>participate</u> in up to 6 repellency tests for up to 42-16 hours duration. In total each of the three products is to be tested for 42-up to 14 hours at each of the two field sites. Each participant will be followed up after each visit by email/in person/by phone within 72-48 hours of the visit to monitor for and asked whether they experienced any unreported adverse events. The minimum duration of participant involvement would therefore be approximately <u>up to 4-6 days 1 week (1-day consent, 1-day training and attractiveness test, 1-day field testing, up to 48 hours post-field testing monitoring).</u></p> <p>Total study duration (recruitment and participant involvement) is anticipated to be ~ 6 months.</p>

Commented [AM4]: Revise period for monitoring adverse effects to 48 hours to align with a 48 hour "wash out" period between exposures for studies where a participant is enrolled for more than one day of testing.

Inclusion Criteria:	<ul style="list-style-type: none"> • Able and willing to give fully informed consent; • Able to understand and comply with the study procedures; • Consider themselves to be in good general health; • Male or female; • Aged 18 to 55 years; • Non-smokers or willing to refrain for 24 hours prior to and during each test; • Willing to undergo a mosquito attraction test putting an arm into a cage of mosquitoes; • Willing to complete mosquito handling training and complete dose determination assays. • <u>Able to withstand exposing the lower leg to mosquitoes for periods of at least 5 minutes at a time</u> • <u>Able to operate an aspirator</u> • <u>Able to speak and</u> understand English
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Exclusion Criteria:	<ul style="list-style-type: none"> • Suspected or known to be sensitive or allergic to, or phobic of, mosquito bites; • Participated in an interventional study (other than an insect repellent study) in the previous 3 months; • Participated in a biting insect study in the previous 48 hours; • Aware of having any cardiovascular or respiratory disorder (whether active or inactive); • Individuals with localized skin disorders or problems affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes; • Allergic to any of the test or reference product ingredients; • Women who are pregnant, nursing or intending to become pregnant; • Previous anaphylaxis; • Aware of having a compromised immune system; • <u>Employees, managers, and spouses of employees of the University of Florida and of the study Sponsor-</u> • <u>Students of the primary investigator or any other University of Florida faculty/researchers involved in the study</u> • Unable to <u>speak and</u> understand English
Efficacy Endpoint(s):	Primary: Median Complete Protection Time for the each repellent product; tested against mosquitoes for prevention of landing.
Safety Endpoint(s):	Adverse event data will be collected and summarized.
Statistical Methods:	Median Complete Protection Time will be calculated using the Kaplan Meier survival-function.
Investigation Site(s):	Single-center: Entomology and Nematology Department, University of Florida, PO Box 110620 Building 970, Natural Area Dr. Gainesville, FL 32611.
Monitor:	Arthropod Control Product Test Centre (<i>arctec</i>), Chariot Innovations Limited, a wholly-owned subsidiary of the London School of Hygiene & Tropical Medicine (LSHTM).

Commented [AM5]: Amend to include proposed and any alternate field testing locations

Commented [AM6]: LSHTM/UF have indicated they will replace the monitor with one that is completely independent of the study.

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Commented [AM7]: Suggest adding a section on "Recruitment" immediately following "Alternatives to Human Study Research". See draft text

Commented [AM8]: Revise to match revised numbering.

1. Introduction

Mosquitoes, midges, sandflies and other biting insects are vectors of extremely important diseases such as malaria, yellow fever, filariasis and many viruses and also may be of great nuisance value. The use of repellent products can provide added personal protection from disease transmission and nuisance bites. New effective repellents would offer an additional option for protection against biting insects. The tests carried out provide important information on the effectiveness of skin repellents, which will be used for label claims to accurately inform consumers and registration purposes.

The aim of this study is to provide longevity and efficacy data for three topically applied insect repellent products for prevention of mosquito landing. The product will be provided by LivFul Inc.

2. Objectives

Primary objective: To determine the efficacy and duration of three topically applied insect repellent products at preventing landing by mosquitoes over 12 hours.

Commented [AM9]: If the expected duration of the repellent's efficacy is >12 hours, EPA suggests that you consider lengthening the test period to ensure the data reflect CPT.

3. GLP Compliance and Quality Assurance

Good Laboratory Practices, as defined by 40 CFR part 160 will be followed throughout this study.

A representative of [entity to be named] independent Quality Assurance Unit (QAU) will perform all QA duties. The QA representative will conduct critical phase inspections at intervals adequate to ensure study integrity, and maintain written and signed records of each inspection. Records shall identify the study and include the date of the inspection, the phase inspected, the individual conducting the inspection, positive and negative findings, actions recommended and taken to resolve negative findings, the scheduled date for re-inspection (if any), and the date(s) the findings are reported. All inspection findings will be reported to management and the Study Director. Any problems, amendments or deviations discovered shall be brought to the attention of the sponsor, Study Director and management immediately. The QA representative will review the final reports for accuracy and compliance with GLPs and the protocol. A signed QA statement will be included in the final report that lists the phase inspections that were conducted, their dates, and the dates the findings were reported to management and the Study Director.

Commented [AM10]: This is sample GLP language. Please edit as appropriate to reflect the GLP/QA plan for this study.

4. IRB Review and Ethical Study Conduct

The protocol, informed consent materials, and other supporting information must be submitted to an Institutional Review Board (IRB) for review and must be approved before any portion of the study is initiated, including recruitment of the subjects. To maintain scientific integrity in regards to testing procedure and clarity of the protocol, any revisions

made to the protocol as a result of the protocol review process will be reflected directly in the protocol itself.

All amendments and deviations will be reported to the study sponsor in a timely manner. All amendments and deviations to the protocol will be reported to the IRB consistent with their standard reporting guidance. Protocol amendments may not be initiated without prior IRB review and approval except where necessary to eliminate apparent immediate hazards to human subjects.

All amendments, deviations, and any adverse events will be documented in the final study and reported consistent with IRB reporting procedures. Documentation will include a description of the change, the reason for the change, the effect of the change on the conduct and outcome of the study, and whether or not the IRB approved each amendment prior to implementation.

This trial will adhere to the principles outlined in the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations. In addition, the study will be conducted in accordance with the ethical principles originating from the Declaration of Helsinki and that comply with current applicable regulatory requirements. They will follow EPA's Product Performance Test Guidelines OPPTS 810.3700: Insect Repellents to be Applied to Human Skin. These studies will be conducted in accordance with all applicable laws, regulations, and IRB requirements.

The IRB responsible for approval and continuing review of this research is:
University of Florida IRB-01
P.O. Box 100173
Gainesville FL 32610-0173

35. Study Design

This is a single-center, two-site field study with all participants testing at least one product at one site. The control for each test is ~~an~~ will be two untreated persons. Repellent product testing ~~takes~~ will take place in a field setting using ~~401313~~ 13 test subjects participants (with a 50:50 ratio of males to females ~~preferably 6 males and 7 females or vice versa~~, with a minimum ~~of three~~ ~~five~~ of five of either gender) for mosquitoes. Each participant will test a single product at a time (see section 8). All testing of a single product at a single site will occur during one test period (i.e., one day). Two untreated participants will also monitor the landing rate at each test site for each test substance throughout the study. An additional 6 participants will be recruited and enrolled as alternates for each test day and will need to be present at the start of each test day in the event they are needed to replace an enrolled subject.

35.1. Study Endpoints

The primary endpoint is the median Complete Protection Time (CPT) of the repellent product. The CPT is defined as the time between application of the repellent product and the occurrence of the first landing in a ~~5-minute~~5-minute test, followed by a confirmatory landing within 30 minutes, or two landings within the same 5-minute test.

35.2. Risks and Benefits

Participants will be exposed to biting insects and if bitten may experience some irritation and itching.

- The study sponsor will provide topical, over-the-counter anti-itch cream at the conclusion of a test day upon request to anyone who is experiencing irritation and/or itching as a result of his or her participation in the study.

As this is a field study and subjects may be exposed to mosquito bites that could transmit vector-borne diseases, efforts will be taken to minimize the risk of infection.

- Mosquito sampling will occur in the two test sites weekly for a month prior to the tests using two trap types. aAll mosquitoes captured will be identified and select genera will be submitted for pathogen testing.
- This is a landing study and mosquitoes will be captured before they have chance to bite.
- All participants will be trained in aspirating mosquitoes and spotting mosquito landing behavior.
- All participants will be asked to wear light, loose fitting clothing that fully covers ~~the rest of~~ their body. They will also be provided with and directed to wear a head net and gloves during the periods of exposure to biting insects.
- All mosquitoes that land will be captured, identified and select genera will be submitted for pathogen testing.
- All *Culex* species captured will be tested for West Nile virus (WNV).
- All *Aedes* species captured will be tested for Zika virus (ZIKV).

Subjects may experience risks from exposure to the test substance, IR3535. According to EPA's risk assessment, All three products contain IR3535, which is moderately irritating to the eyes, but is not irritating to the skin ~~based on dermal studies in rabbits and human volunteers~~. It may also produce an allergic reaction or irritation of the respiratory tract.

- To protect against the risks associated with use of the product, correct handling by the researcher will apply the product to test subjects in a manner that will avoid risks associated with exposure of the eyes or respiratory tract.
- Volunteers will ~~also~~ be excluded if they have a known allergy to any of the product ingredients, or any skin condition which-that may affect their reaction to the product.

Commented [MD11]: Run-on sentence

Commented [FC12R11]: Needs to be rewritten:
Weekly mosquito sampling will take place during a month prior to test initiation. Sampling will be conducted using traps. Trapped mosquitoes will be identified to genera and submitted for pathogen testing.

Commented [AM13]: Confirm that these are the only disease vectors that could be transmitted in the test areas. If other vector-borne illnesses could be present, amend protocol to include testing a subset of collected mosquitoes for all potential vector-borne illnesses.

Commented [AM14]: Please delete this reference. You can cite EPA's risk assessment for IR3535 if necessary.

There is insufficient evidence to fully characterize the risk of IR3535 to pregnant or lactating women. Therefore, pregnant women or women intending to become pregnant will not be included in the study.

Commented [AM15]: EPA regulations prohibit any research involving intentional exposure of pregnant or nursing women, so regardless of the risk, research with this population is not permitted.

Other risks associated with participation in this trial include the risks of associated with being outside in a hot humid climate, such as sunburn, heat stroke. In addition, there is a risk of fatigue due to the length of the test day.

- Precautions will be taken to prevent sunburn, exposing only minimal skin, wearing a hat etc. Participants will be directed to spend the time between test periods in a covered pavilion/tent with screen doors/walls, etc....
- Water and other drinks will be provided to prevent dehydration and snacks will be provided to maintain blood sugar levels if necessary.
- Subjects will be told at the consent meeting and reminded at the training to bring snacks, lunch, and entertainment to occupy their time during breaks between test periods.
- Subjects will be provided with breaks as needed between the test periods. Chairs and a shaded area will be provided for relief from the sun. Subjects will have an opportunity to eat lunch, dinner, and snacks, and will have opportunities to use the restroom as necessary.
- In addition, any time remaining immediately following an exposure period and before the start of the following exposure period, subjects will be encouraged to stretch and walk around as needed to try to minimize the discomfort from the length of the testing period, as well as to remain inside the shaded and/or screened area to avoid heat-related illnesses.
- Where a subject enrolls to participate in more than one test day, at least 72 hours will lapse between each test day to allow subjects to rest and recover.

A potential risk of participation is unintentional release of confidential information.

- All efforts will be taken to maintain subjects' confidentiality. See the precautions in Section 11.3, "Confidentiality".

Commented [AM16]: Numbering to be updated

There can be psychological stress relating to pregnancy testing.

- In order to minimize the psychological stress, women will be given a private place to take the test, a female member of the study team will verify the test result, and the study director will ensure confidentiality of any test result. The results of the test will not be discussed with or released to anyone besides the subject. The confidentiality of the pregnancy testing will be discussed during the consent process.

General risks to participants associated with involvement in this study will be addressed by adhering to ICH GCP², the Declaration of Helsinki³, the Data Protection Act⁴ and all applicable regulatory requirements.

Commented [AM17]: See comments on references section about these citations.

There will be no direct benefit to participants. Indirect benefits to society will be additional products available to consumers to repel mosquitoes, thereby reducing the potential for mosquito bites and transmission of vector-borne illnesses. improved products for prevention of mosquito biting and pathogen transmission. The results of this study will inform the product labelling.

3.3 Alternatives to Human Study Research

This study will use human subjects because no reliable models or surrogates have been found to adequately predict the efficacy of topically-applied insect repellents.

As the objective of this study is to determine the efficacy of a repellent in protecting human beings against bites from mosquitoes, it is necessary to complete this testing using human subjects. As human subjects are known to provide a complex combination of thermal, visual and olfactory cues that are attractive to mosquitoes looking to bite and feed, an alternative model is not currently available that will test the repellent in a suitably realistic scenario. The repellent must repel the mosquito in the presence of the attractive host in order to be truly effective. The risks of this research and the steps taken to counteract the risks are described in section 3.2. Every effort will be made to protect the subjects in this study from all potential hazards. Products containing IR3535 have been registered by EPA, and the risk assessment has shown that this active ingredient presents little or no hazard when used as directed.

4. Participant Entry

4.1. Screening Procedures

Volunteers will be consented prior to any screening procedures being undertaken. Female volunteers of child bearing potential will be asked to take a pregnancy test at the consent meeting (?), training session, and at the beginning of each test day in which they participate. Volunteers who do not meet the criteria for eligibility will be excluded.

Individuals who express an interest in participating in response to the recruitment materials will be contacted by telephone or e-mail (in which case a follow up telephone call will be made) to determine whether they meet the basic inclusion criteria. They will be given a brief outline of the study. If they are interested in enrolling in the study, they will be given a time, date and location to meet with University of Florida staff for a training session to learn more about the study and their potential role in it, go over the inclusion/exclusion criteria and the informed consent materials, receive answers to any questions they may have, and to provide informed consent to participate in the study. Contact information is included on the consent form for any individual who has additional questions or if further clarification is desired, after they have attended the training session.

Commented [AM18]: Note that candidates will be screened by phone using a script (at), how/when they will be brought in for the consent meeting

Commented [AM19]: Who will make the contact? The study director, study personnel, etc?

Commented [AM20]: Again, explain who will conduct the initial training session/consent meeting.

4.2. Inclusion Criteria

Volunteers will be healthy individuals and chosen based on their insensitivity to the bites in order to limit any itchiness or discomfort.

Volunteers will be ~~included-eligible to participate~~ in the study if they meet all of the following criteria:

- Able and willing to give fully informed consent;
- Able to understand and comply with the study procedures
- Male or female;
- Aged 18 to 55 years;
- Non-smokers or willing to refrain for 24 hours prior to and during each test
- Willing to undergo a mosquito attraction test (putting an arm into a cage of mosquitoes)
- Willing to complete mosquito handling training
- Able to stand outside for periods of at least 5 minutes at a time
- Able to operate an aspirator;
- Able to speak and understand English

Commented [AM21]: Match with revised inclusion criteria in opening table

Volunteers will be advised not to apply any cosmetics associated with a strong scent, such as perfume, hand cream, body wash, or scented shampoo for the 24 hours immediately preceding the study. Additionally, volunteers will be asked not to drink alcohol or consume spicy foods, i.e., curries, hot peppers and garlic, and to not engage in vigorous exercise for the 24 hours prior to ~~the each tests~~. This A study staff member will verify each subject's compliance with this request on each test day prior to performing any treatment with a test substance. will be verified with the participants prior to the commencement of any tests.

Due to the nature of the study, a field study, it is important that all instructions are understood fully and quickly. Therefore, it is essential that the participants understand English. If the study staff are concerned that the participant is not proficient enough in the English language they will not be permitted to ~~continue enroll in the study~~. Current repellent product labels are in English and the language that someone speaks does not directly affect attractiveness to mosquitoes. To target users familiar with and that understand the product labels, we will be recruiting English speaking subjects. This research does not offer benefits to the subjects, so limiting recruitment to English speakers will not result in equity-of-access issues

Commented [AM22]: Please move this to the study withdrawal section.

4.3. Exclusion Criteria

Volunteers will be excluded from the study if they meet any of the following criteria:

- Suspected or known to be sensitive or allergic to, or phobic of, mosquito bites;
- Participated in an interventional study (other than a biting insect challenge study) in the previous 3 months;

Commented [AM23]: Match with revised exclusion criteria in opening table

- Participated in a biting insect challenge study in the previous 48 hours;
- Diagnosed with any cardiac or respiratory disorder (whether active or inactive);
- Individuals with localized skin disorders affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes;
- Allergic to any of the test or reference product ingredients;
- Women who are pregnant, nursing or intending to become pregnant;
- Previous anaphylaxis;
- Aware of having a compromised immune system;
- Employees, managers, and spouses of employees of the University of Florida and of the study Sponsor;
- Students of the primary investigator or any other University of Florida faculty/researchers involved in the study
- Unable to speak and understand English
- Unable to aspirate mosquitoes

4.4. Withdrawal Criteria

Participants can withdraw at any time without giving a reason for withdrawing and without forfeiting benefits based on their participation prior to withdrawal. Data collected to the point of withdrawal will be used in the analysis of the study, unless the participant requests that their data is not used, in which case it will be removed from the database. Participants may also be removed at the discretion of the Principal Investigator, where continued participation may affect the safety of the participant or where there is a development of any condition which might interfere with study participation.

5. ~~Randomization and Enrollment~~ Enrollment and Assignment to Treatment, Control or Alternate Groups

5.1 Enrollment

Subjects will be enrolled to participate in the study following the process described in Section 8.3. Volunteers will be fully informed before the study and it will be made clear that they can withdraw from the study at any time and without forfeiting benefits based on their participation prior to withdrawal. Volunteers will be given and asked to read the consent form which must be signed before the test begins. Subjects will be eligible to enroll in more than one test day, but at least 72 hours must elapse between test days involving the same subject.

5.2 Randomization

For each test day, the ~~the~~ 15 subjects will participants will be randomly assigned to either a treatment or untreated control group. 15 pieces of folded paper will be placed in a box, two pieces of paper will have the word "control" and thirteen will have the word "treatment". Will/With reference to the participant list, the PI will draw the pieces of paper in order to assign treatments to participants.

Commented [AM24]: Indicate how a withdrawal during the field trial will be handled in light of the group transportation from the lab to the field testing site. Will transportation back to the lab be provided immediately? If so, by whom? Will the subject withdrawing have to find his/her own transportation back? Who will step in to handle aspirating mosquitoes for the partner of the subject withdrawing?

Commented [MD25]: Just a comment - from a stats point of view, this is fine and we can just call that data right-censored if they withdraw earlier prior to bite. Data CAN still be included up to that point and the stats will appropriately consider it.

Commented [AM26]: Protocol should also explain how subjects will be assigned to the test or alternate group. Order of enrollment or random assignment prior to assignment to test/control groups?

Commented [NJ27]: This section was copied from section 7. Treatment. Updated numbers were suggested to reflect new sample size.

6. Adverse Events

6.1. Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or study participant
Serious Adverse Event (SAE)	<p>A serious event is any untoward medical occurrence that:</p> <ul style="list-style-type: none"> Results in death Is life-threatening Requires inpatient hospitalization or prolongation of existing hospitalization Results in persistent or significant disability/incapacity Consists of a congenital anomaly or birth defect <p>Other 'important medical events' may also be considered serious if they jeopardize the participant or require an intervention to prevent one of the above consequences.</p>

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Commented [AM28]: Clarify who will be exercising the medical judgment and how they are qualified.

6.2. Reporting Procedures

All adverse events and serious adverse events should be reported. Depending on the nature of the event the reporting procedures listed below should be followed. Any questions concerning adverse event reporting should be directed to the Principal Investigator in the first instance.

6.2.1. Non serious AEs

All adverse events should be recorded on the Adverse Event Record Form (Appendix 1) and Adverse Event Monitoring Questionnaire (Appendix 2) and entered into a spreadsheet and stored on a secure drive. All adverse events will be reported to the Sponsor. Depending on the nature of the event the reporting procedures below should be followed, see Appendix 3 for a flowchart of safety reporting.

6.2.2. Serious AEs

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the definitions in section 7.1. AE questionnaires meeting the SAE definition will be submitted to the Principal Investigator, Dr Emma Weeks within 24 hours. SAEs that are assessed by the Principal Investigator as being both related and unexpected must be reported to the UF IRB within 5 days of the Principal Investigator becoming aware of the event.

In the case of a severe reaction such as anaphylaxis, a trained First Aider will be called immediately and the volunteer taken to the nearest Emergency Room (ER).

7. Treatments

LivFul Inc. will provide the three products to be tested. The three products have the same active ingredient, IR3535, in a spray, lotion or wipe. ~~Dose determination will be conducted to obtain the typical consumer dose, prior to test commencement (see section 8.4.3). For each test day, the product will be applied to the participant's lower leg of 13 test subjects. The dose will not exceed the maximum daily limit for IR3535 (see Appendix 4 for calculations of the maximum safe dosage of IR3535). In addition, for each test day, two subjects will not be treated and will serve as negative controls will be completed at each time point to monitor mosquito landing rates (see section 8.5.2). The participants will be randomly assigned to either a treatment or untreated control group. Twelve pieces of folded paper will be placed in a box, two pieces of paper will have the word control and ten will have the word treatment. With reference to the participant list, the PI will draw the pieces of paper in order to assign treatments to participants.~~

8. Test Methodology

8.1. Field Sites

Field tests for mosquito repellents will be conducted in at least two distinct habitats, most likely a forest or wetland and an urban environment, where the predominant mosquito species differ. The test will most likely be conducted in Alachua county, Florida, USA. This area is outside the current hotspot of ZIKV transmission but in an area of high mosquito abundance and diversity. However, efforts will be made to include a site where *Aedes albopictus* is present.

Commented [AM29]: If risk of anaphylaxis is low/negligible, be explicit about the potential risk and note that epi pens would be on-site, first aider/nurse would be on site, etc. and if an incident occurred, the person would be taken immediately to the ER.

If no/negligible risk of anaphylaxis, note that risk is negligible, first aider on site sufficient to respond to incidents that occur during the study

Commented [AM30]: A trained/certified first aid person should be on site for all test days. Protocol should note that first aid will be rendered on site if necessary and ED will be called if necessary.

Commented [BE31]: The Agency recommends using a dose of 1 g product/600 cm² for all products except pump sprays. EPA recommends testing pump sprays at 0.5 g product/600 cm². These numbers are based on an analysis of dosimetry results from repellent studies reviewed by EPA and HSRB since 2006.

Commented [AM32]: Who decides which lower leg? If subjects participate in more than one test day, will the treatment be applied to the same leg, different leg, or randomly assigned on each test day?

Commented [AM33]: Suggest incorporating additional recommendations from HSRB

- Monitoring > 4-12 weeks prior to field testing
- No cases of diseases identified within 4 weeks of testing
- Outline how you will coordinate with local health departments/mosquito control districts to learn of incidences of diseases occurring in testing area
- Conduct testing in areas with active vector-borne illness monitoring programs

<https://www.wuft.org/news/2016/05/27/mosquito-trapping-begins-for-the-summer-season/>

Commented [AM34R33]: Also consider listing additional potential test sites in this protocol so you can address issues of coordination with local mosquito abatement districts, etc. without amending the protocol down the line

8.1.1. Site monitoring

Potential sites for testing mosquito repellents will be monitored at least weekly for a month before testing is scheduled. Two trap types will be utilized, a CDC light trap and a BG Sentinel trap. Trapping will occur for 24 hours and mosquitoes captured will be processed as described in section 8.1.2. To minimize risks to subjects, field testing will not be conducted where WNV, ZIKV, dengue, chikungunya or other mosquito-vector diseases have been detected within the previous two-four weeks. The sites will also not be known ZIKV transmission areas.

Commented [AM35]: Add to this section monitoring by the local health department/mosquito abatement district, how coordination will occur, etc.

8.1.2. Mosquito processing

Mosquitoes captured in the pre-test sampling period will be identified by genus and species, and if possible, by subspecies or strain. *Culex* species will be tested for WNV and *Aedes* species will be tested for ZIKV as described in section 8.1.3. Mosquitoes will be pooled by study site and sampling date into groups of 10 by genera and tested following established protocols.

Commented [AM36]: To minimize risks to subjects, ensure you are testing for all possible vector-borne illnesses that could be present in the area, e.g., dengue and chikungunya.

8.1.3. Mosquito pathogen testing

The adult *Aedes* species mosquitoes from field collections will be analyzed to detect ZIKV RNA using reverse transcription and quantitative PCR (RT-qPCR). In a biological safety cabinet, legs from each mosquito body will be removed and placed in clean individual tubes and stored at -80°C for later processing. The RNA will be extracted from each mosquito body using QiAmp viral RNA kit, whose reagents have been shown to inactivate viruses. RNA mixed from pools of 10 bodies will be screened with qRT-PCR reaction using the iTaq™ Universal Probes One-Step kit (BioRad). Primers and a probe specific to ZIKV are designed to the NS2B gene of the ZIKV isolate (accession #KX520666). A mosquito will be considered positive for ZIKV RNA if qRT-PCR reactions show a Cq ≤ 36.⁵ Samples that are positive for ZIKV RNA will be further validated using RT-qPCR to amplify the same NS2B gene and sequence analysis performed (Eurofins MWG Operon LLC). If warranted and time and money permits, RNA from individuals in the positive pools can be screened for presence of ZIKV to estimate infection rate of the field collections.

The adult *Culex* species bodies from field collections will be analyzed to detect WNV RNA using already established protocols.⁶

8.2. Test Insects

Mosquito tests will be conducted where more than one species are present. A site will be selected that has an abundance of ZIKV vectors (*Aedes albopictus*), but no previous history of transmission (see section 8.1.1). During each test period, l-landing insects will be aspirated or trapped before and during the test, and labeled with the time of collection. After the field study, collected insects will be identified by genus and species, and if possible, by

Commented [BE37]: This is covered above in the pre-test monitoring and doesn't make sense in the context of this sentence. We should make sure we have the landing insects separated from trapped insects for data collection and review.

subspecies or strain. The number in each taxon collected in each time period will be reported.

After identification, mosquitoes will be subjected to analysis to determine the presence or absence of WNV, ZIKV or other disease organisms as described in section 8.1.3. The results of these analyses will be reported to subjects and included in the study report.

Commented [AM38]: Include information about how you will follow up with test participants if any of the screenings come back positive.

8.3. Volunteer enrollment

8.3.1. Recruitment of volunteers

Recruitment will not begin until EPA and the HSRB have reviewed the protocol and the associated informed consent document, these documents are revised to address comments from EPA and the HSRB, and the IRB has approved the final versions of these documents. For each test substance tested, there will be thirteen test subjects (at least five of each gender) and two untreated control subjects selected per testing period. There will be at least 6 alternates per test day who will need to be present on test days until the researchers determine whether they are needed to replace an enrolled test subjects. All subjects will be 18 to 55 years of age.

[insert description of advertisement, when/where it will be posted, how people can use the information on the poster to enroll in the study, etc.]

Subjects will be recruited from the Gainesville, Florida area, via advertising through digital and social media. Advertisements will be posted in digital and social media mediums, such as Facebook, Yahoo/Bing, Google and Craigslist. Every effort will be made to achieve the demographic composition, via a stratified random sample of the pool of recruited subjects. The qualifying subjects will be stratified into smaller subgroups according to their race/ethnicity, age, and gender to help ensure that the subjects are as representative as possible of the general population of skin-applied repellent users. The final report will specify the demographics of test subjects who participated in the study, taking into account the availability of test subjects on each test day.

Current repellent product labels are in English and the language that someone speaks does not directly affect attractiveness to mosquitoes. To target users familiar with and that understand the product labels, we will be recruiting English speaking subjects. This research does not offer benefits to the subjects, so limiting recruitment to English speakers will not result in equity-of-access issues.

Following approval review by the local Ethics Committee and EPA and the HSRB, and after IRB approval of the protocol that addresses all comments from EPA and the HSRB, at least 20-2430 informed and consenting volunteers will be enrolled to take part in the study

via an approved recruitment email/poster/advert. From this pool of eligible subjects the participants will be selected to be representative of age, gender, race/ethnicity of the general population. ~~Ten~~13 informed and consenting participants (at least 5 of each gender ~~3 males and 3 females for each test site and product~~) will be recruited. In addition, ~~2XX~~ untreated control participants for each test site will be recruited. There will also be ~~six~~? alternates, for a total number of participants of ~~48~~???. Only participants who are not sensitive to mosquito bites will be involved in this experiment and those taking part are free to withdraw at any time. Up to an additional ~~6~~?? people could be enrolled as alternates.

Commented [BE39]: Approximately 50/50; 6 males and 7 females or vice versa

8.3.2. Consent and screening

After the screening is complete, and prior to participating in any study-related procedure, each potential subject will meet in person, either individually or in small groups, with the Study Director for a consent meeting.

During the consent session, the following aspects of the study will be discussed and the following activities completed:

1. Upon arrival, subjects will be asked to provide proof of age with a driver's license, passport, or other valid identification.
2. Subjects will be given the Informed Consent Document (ICD), time to read the ICD, and the opportunity to ask questions about it. The trainer will provide a brief outline of the study including its purpose, the subjects' potential role in the study, the potential length of the study on any given test day, the identity and function of the pesticide to which they will be exposed, the potential hazards associated with the study and steps being taken to mitigate each hazard as addressed in the protocol, and the inclusion/exclusion criteria. The procedures involved with the attractiveness test, training on aspirating mosquitoes, and a 5-minute exposure interval will be explained and demonstrated step-by-step to all subjects who participate in the training. The subjects will be shown how the test substances will be applied to their leg for the future testing as per section 8.5.4 of the protocol, will be informed that they will wear gloves to protect their hands and head nets to protect the head, face and neck, and will be shown how to aspirate mosquitoes.
3. Any questions or concerns about the study will be discussed and answered.
4. The employee conducting the consent session with test subjects will let all training attendees know that if a test subject needs to speak to the study director in private about any aspect of the study, time will be made for this discussion once the general consent session is over.
5. To confirm understanding of the consent form, the following questions will be asked:
 - a. What part of your body will be treated and what will be used in this study?
 - b. What will you be wearing during the exposure period?
 - c. How long will you be exposed to mosquitoes for during the field test for each exposure?

Commented [AM40]: The documentation provided to the IRB indicates that the study director, Dr. Weeks, is the only party who will be responsible for obtaining informed consent. If you intend to have other study personnel involved in the consent process, please indicate here and note how they are qualified.

Commented [AM41]: Need to add a section on the consent meeting for volunteers – what it will cover, who will conduct it, how long it will last, etc.

- d. What are the potential discomforts or hazards from this study?
- e. Do you have the freedom to quit or withdraw from the study at any time?
- f. If you quit or withdraw from the study, for how many hours will you be paid?
- 6. The trainer will recommend that subjects bring their own form of entertainment (book, DVD player, computer, etc.) to minimize participant anxiety and potential boredom during testing procedures. The researcher will have drinks (e.g., bottled water, soft drinks, etc.) and snacks available for subjects during the study day. Researchers will ask subjects if they have any food allergies and make snacks available taking into account the responses. Subjects will be told that they can bring their own lunch and snacks to consume during a break between exposure periods.
- 7. The trainer will provide test subjects with the study director's contact information (name, email, and phone number) to field any follow up questions. This information will be on the first page of the provided ICD.
- 8. Female participants will be notified that they will be required to undergo pregnancy testing at the beginning of each testing day.

Commented [AM42]: Suggest including questions to ensure that people understand the content of the ICD before providing consent.

The potential subject will be given ample time to ask and have all questions answered.

If an individual still wishes to enroll in the study, he or she will be asked to sign the ICD, which will be witnessed by the staff member who led the consent discussion. Their eligibility to take part will then be assessed using a participant-completed questionnaire to screen for confounding health conditions that may make them unsuitable for taking part. For females, a negative pregnancy test prior to the training day and every test day is required in order to enrol and maintain enrolment of such a participant. All females will need to confirm that they are not pregnant and do not intend on becoming pregnant throughout the course of the study. See section 8.4.1. for more details. The subject will then be given a photocopy of the signed ICD and testing schedule, and scheduled to attend a mosquito attractiveness test and training session.

~~As part of fully informed consent, the participants will review the procedures associated with each exposure interval during the study so they fully understand what will be expected of them on the test day. The following topics will be covered, the content of the informed consent form, provide an outline of the study, discuss subjects' role in the study, discuss the identity and function of the repellent to which they will be exposed, the potential hazards and steps being taken to address them, and the inclusion/exclusion criteria.~~

~~Following a detailed review of the participant information sheet and face-to-face meeting, informed volunteers will be asked to provide their written consent to take part in the study. Their eligibility to take part will then be assessed using a participant-completed questionnaire to screen for confounding health conditions that may make them unsuitable for taking part. For females, a negative pregnancy test prior to the training day and every test day is required in order to enrol and maintain enrolment of such a participant. All~~

~~females will need to confirm that they are not pregnant and do not intend on becoming pregnant throughout the course of the study. See section 8.4.1. for more details.~~

8.4. Pre-test participant preparation

8.4.1. Pregnancy testing of females

Female subjects must not be pregnant or be breast-feeding. To confirm that participating test subjects are not pregnant, at the beginning of any day when they will be exposed to mosquitoes, female subjects will be required to perform an over-the-counter pregnancy test that will be supplied by the University of Florida. Each female test subject alone, in a bathroom at the testing site, will perform the test. The test subject will initially see the results only. After completion of the pregnancy test, a female employee associated with the study will ask, in a private setting, if the potential subject still wants to participate in the study. If they do, the negative test result will be verified by that employee and relayed to the Study Director (if the Study Director is not the verifying employee). The results will be kept confidential, and will not be disclosed to anyone other than the test subject, the verifying employee, and/or the Study Director. Test subjects will not be required to disclose the results of the test, with the understanding that if they do not, they will not be allowed to participate in the test. Provisions will be made to allow the test subject ~~will to~~ dispose of the test results in a discrete manner (e.g., opaque plastic bags available in the restroom used for testing). This procedure will be repeated for each test day in which any female subject participates.

8.4.2. Attractiveness test

After completing the consent process, ~~Each participant's will be tested for~~ attractiveness to mosquitoes will be verified before they proceed further in the study. They will place one arm in a 45 x 45 x 45 cm cage of 78 mosquitoes (*Aedes*) or density equivalent to one mosquito per 1,160 cm³. If they do not receive five landings in one minute they will be considered not to be sufficiently attractive to mosquitoes and will not be allowed to continue with the study or finish the training. Pathogen-free colony insects will be used that have been in colony for at least 10 years. They will be tested prior to the study to confirm the absence of ZIKV (*Aedes*) and WNV (*Culex*). ~~Pathogen-free colony insects will be used that have been in colony for at least 10 years. The mosquitoes will be tested prior to the study to confirm the absence of ZIKV (*Aedes*) and WNV (*Culex*) as described in section 8.1.3.~~

8.4.3. Insect landing catch training

Participants will be trained in a screened free-flight cage to identify mosquito landing behaviour and to use aspirators to collect landing insects before they have time to probe or bite. Pathogen-free colony insects will be used that have been in colony for at least 10 years. The mosquitoes will be tested prior to the study to confirm the absence of ZIKV (*Aedes*) and WNV (*Culex*) as described in section 8.1.3.

Commented [AM43]: Does this occur at the same meeting as the consent process? Is there a separate visit to the center? How soon after the consent process does this need to occur?

Commented [AM44]: Have these mosquitoes had a blood meal?

Commented [AM45]: When and where will this occur? How long will it take? How soon after the consent meeting and attractiveness test?

Include more about the process for the training, how you will determine that a person is sufficiently capable of aspirating mosquitoes in the field to be finished with the training process, etc.

Commented [AM46]: EPA suggests that subjects be protected from mosquito bites during this training by wearing long sleeved shirts, long pants, and being provided with head nets and gloves.

8.4.3. Dose determination

To estimate a “typical consumer dose” the application of the three products will need to be evaluated prior to the study. Participants allocated to the negative control group will not be involved in dose determination. To estimate a “typical consumer dose” each participant will be asked to apply the repellent lotion or wipe three times to themselves as they would do normally to cover their leg evenly from the top of the sock to the knee. The surface area of the leg also will be calculated. The amount applied in each application by each participant will be calculated by weighing the bottle or wipe before and after application. For the spray, the legs of the participant will be wrapped in gauze “bracelets” of a known area. The bracelets will be weighed before and after application of the spray. The “typical dose” in mg/cm² converted to ml/cm² skin will be calculated as the mean of three applications by each of the participants and the specific gravity of the test material.

Commented [BE47]: As discussed in our conference call we recommend the standard doses of 1 g product/600 cm² for all but pump sprays and 0.5 g product/600 cm² for pump sprays. Please provide methods for application of standard dose.

To estimate a “typical consumer dose” the application of the three products will need to be evaluated prior to the study. Participants allocated to the negative control group will not be involved in dose determination. To estimate a “typical consumer dose” each participant will be asked to apply the repellent lotion or wipe three times to themselves as they would do normally to cover their leg evenly from the top of the sock to the knee. The surface area of the leg also will be calculated. The amount applied in each application by each participant will be calculated by weighing the bottle or wipe before and after application. For the spray, the legs of the participant will be wrapped in gauze “bracelets” of a known area. The bracelets will be weighed before and after application of the spray. The “typical dose” in mg/cm² converted to ml/cm² skin will be calculated as the exponential of the mean of natural log transformed data of three applications by each of the participants and the specific gravity of the test material.

8.5. Test methodology

8.5.1. Subject meeting

On the test day, sSubjects will meet at the Entomology and Nematology Department, University of Florida, Gainesville FL, or other suitable location for application of the repellent. They will be permitted to leave after treatment as long as they agree to return and in the meantime avoid alcohol, tobacco, and scented products (perfume, cologne, hair spray, lotion, soap, etc.). In addition, participants should avoid strenuous exercise and sweating before and during the study, as well as avoiding abrading, rubbing, touching, or wetting the treated area. Transport will be provided to study sites in preparation for the start of the testing.

Commented [AM48]: In order to ensure that subjects who are treated complete the study and to support the scientific validity of the study/GLP (integrity of the application of the test substance), EPA recommends changing this to require subjects to be on site from the time of treatment through the field testing period.

Commented [AM49]: How will you get a subject who wishes to withdraw from the study once in the field back to the lab? Need to ensure that someone who wishes to withdraw doesn't feel compelled to stay in the field.

8.5.2. Subject preparation

Before treatment, study staff will ensure that no subject has participated in another field test for this study in the previous 72 hours. One pant leg will be rolled up securely and a the

Commented [BE50]: Describe when you will utilize alternates, if participants will be replaced partway through or not.

exposed lower leg (ankle to knee), of each participant will be washed with unscented soap and carefully rinsed and dried. With exception of the treated area, the participant's head, hands, trunk, and limbs will be covered with light-colored material through which insects cannot bite. Study staff will verify that participants should avoid alcohol, tobacco, and scented products (perfume, cologne, hair spray, lotion, soap, etc.) and excessive exercise for at least 24 hours before and throughout the test. In addition, participants should will be reminded to avoid strenuous exercise and sweating before and during the study, as well as avoiding abrading, rubbing, touching, or wetting the treated area, especially by not rolling down the pant leg or sitting with legs crossed. Subjects will be provided with head nets and gloves to protect exposed skin from mosquito bites during the field testing.

Commented [BE51]: This should be chosen randomly

Commented [AM52]: Be explicit that subjects need to wear shorts or roll up pants at start of treatment and leave them up for the duration of the test to avoid abrading/rubbing/compromising the treatment area

8.5.32. Untreated control participants

Two participants per test day will not be treated with a repellent on any limb. Before beginning the test day, study staff will ensure that no subject has participated in another field test for this study in the previous 72 hours. One pant leg will be rolled up securely and the exposed lower leg (ankle to knee) of each participant will be washed with unscented soap and carefully rinsed and dried. For untreated control subjects, the participant's head, hands, trunk, and limbs will be covered with light-colored material through which insects cannot bite, except the prepared lower leg which will be exposed periodically to ensure mosquito biting pressure. Study staff will verify that participants avoided alcohol, tobacco, and scented products (perfume, cologne, hair spray, lotion, soap, etc.) and excessive exercise for at least 24 hours before and throughout the test. In addition, participants will be reminded to avoid strenuous exercise and sweating during the field testing. These participants will be fully clothed to prevent mosquito bites.

Untreated control participants will monitor the mosquito activity at regular intervals during the test, by counting and collecting mosquitoes landing on their clothing, to confirm continued acceptable landing pressure as described in section 8.5.5. Control subjects with negative inert substances will not be done to reduce risk to the participants of mosquito biting and pathogen transmission. A positive control will also not be completed because IR3535 is a known efficacious repellent, in this study we are trying to determine the effectiveness (CPT) of the formulations IR3535.

Commented [BE53]: Normally landing counts for the control treatment are on exposed skin, they would expose a similar area of skin and the landing counts are collected on that. After receiving 5 lands, the pant leg could be rolled down to cover the exposed skin to protect from bites (time to reach 5 landings should be recorded). Mosquitoes landing elsewhere could be collected for ID purposes.

8.5.43. Initial landing pressure

As participants will be treated two hours before the start of the test the initial landing pressure will be monitored by the untreated control participants. At least one mosquito landing within one minute should be recorded for the trial to progress.

Commented [AM54]: Please clarify whether this occurs immediately before the first exposure period for test subjects, or at the start of the whole test day (i.e., immediately following application to the test subjects).

8.5.45. Product application

The surface area of the lower leg (ankle to knee), will be calculated in order to calculate how much repellent each participant should receive. The “typical consumer dose” of the lotion or spray will be applied to the lower leg. With the wipe, the applied amount will be measured by weighing the wipe before and after use. Study staff will apply a standard dose to the lower leg of each participant. The repellent will be spread evenly over the lower leg from the ankle to the knee using a single gloved finger to ensure uniform coverage.

8.5.56. Continued landing pressure

After the initial landing pressure monitoring period, before each exposure period starts for the test subjects, the lower limb of each untreated control participant will be monitored (unexposed) for 1 minute to ensure that at least one mosquito lands for the trial to progress. untreated control subjects will expose the lower leg every 30 minutes for 5 minutes or until 5 landings on the lower leg occur, whichever is sooner. As soon as 5 landings occur, regardless of whether 5 minutes have elapsed, the control subject can cover the lower leg to minimize the potential for mosquito bites. The time of the landings and when the threshold number of landings occur will recorded in the study records. Insects-Mosquitoes landing on controls-control subjects will be collected by mouth-aspiration for later identification and labelled with the time of collection.
-aspiration for later identification and labelled with the time of collection.

Commented [BE55]: How many periods will you go before stopping if you have inadequate landing pressure. In previous studies no more than 10% of exposure periods should have less than the minimum landing pressure. How will you handle a landing, followed by another exposure period with no landing and either no landings/below minimum landings in the untreated control. We have treated the first landing as a confirmed landing in that case previously.

Commented [BE56]: Any insects landing on treated subjects should be collected and identified, these should be recorded separately from mosquitoes captured on untreated control subjects

8.5.76. Subject Placement

Each treated and untreated control participant will be paired with a trained member of staff or another participant. The two untreated controls will be paired together. Each pair will be located at least 3 m/10 ft apart from other pairs.

8.5.87. Exposure period

Every 30 minutes during the trial period, the treated lower limb of the treated participantstest subjects will be exposed for 5 minutes. Under supervision of a trained member of staff or another participant, the number and timing of each landing during each exposure period for each participant will be recorded. All landing insects will be collected for identification by aspiration and labelled with the time of collection (before they have chance to probe or bite).

Commented [AM57]: All landing insects anywhere or only on the treated leg?

8.5.89. Exposure duration

In order to minimize subjects' exposure to biting mosquitoes and to ensure an acceptable landing pressure throughout the duration of the study, as well as to preserve wellbeing, maintain morale amongst the volunteers, and work within the limits of daylight available, the first two hours of testing will be skipped. Given the typical range of CPT data for IR3535

Commented [BE58]: Also reduces exposure to biting mosquitoes; we should have at least 3 exposure periods for each subject to occur after the delay to establish a CPT for each individual subject. Also, you should provide steps taken to ensure integrity (e.g., protect from abrasion etc.) of the product from time of application to exposure

products⁷ it is highly unlikely failure will occur before this time point. Each participant will test all time points. Between time points the repellent will be left on the leg and re-tested every 30 minutes up to 42-14 hours or until Complete Protection Time (CPT) has been determined. CPT is defined as one landing in the 5-minute test period followed by a second confirmatory landing in the same period or in the next test period, 30 minutes later, on the treated leg.

Commented [BE59]: Testing for a single product at a single site should be done on the same day for all subjects

Commented [BE60]: Or two landings within the same 5 minute test period

8.5.10. Between exposure periods

Between periods of exposure to mosquitoes for the test and control subjects, the study director will ensure access to a shaded, screened location with adequate seating to accommodate the subjects. In addition, subjects will have access to cold drinks (water, soft drinks, etc.) and snacks to keep them hydrated and to maintain their blood sugar. As discussed during the consent session, subjects may also consume food and drinks they brought, read, or engage in other leisure activities. Between exposure periods, test subjects will be reminded not to engage in activities that could abrade the treated leg (e.g., rolling down pants, crossing legs). The study staff will ensure that subjects have access to restroom facilities when necessary.

8.5.911. Environmental Conditions

The time of day at which subjects are treated and at which exposure to target insects begins and ends will be recorded and reported. Weather conditions (including temperature, relative humidity, cloud cover, precipitation, light intensity, and wind speed) will be monitored periodically throughout the study and reported. Testing will be not be conducted or continued if wind speed exceeds 16 kph/10 mph or if it is raining.

Commented [BE61]: As mentioned in the conference call, we should have a section about weather delays which don't cancel the study. Previously, this was done using the following method: If a confirmed land occurred during the period immediately following the rain delay, the break down period was assumed to be the period when the rain delay began. (e.g., if periods 4 and 5 were delayed/skipped and the first landing occurred in 6 and the confirmed land occurred in 7, then period 4 was used as the failure time for that subject because the confirmed land could have occurred in that period). The skipping no more than 3 periods in a test is acceptable for EPA.

Commented [NJ62]: We did not review or make any comments on this section because it is not included in the objectives of this protocol.

Commented [MD63R62]: This section was apparently not in earlier versions we reviewed and must have been added subsequently. Not clear why this is here or what it does. Not sure why there is a discussion of power. The participants are applying what they normally apply as per earlier description in this protocol. Why is this here and what does it do?

I am ignoring- as this point -whether these have been done properly statwise. Note that these are REPLICATES and this would need to be taken into account.

Finally, there are no units here and also no indication as to what product this is (wipe? Lotion? Etc.). If this were to be used, wouldn't one need to have info for all three formulations?

Commented [MD64R62]: Following internal discussion here at EPA yesterday, my understanding is that this will not be done as part of protocol and instead a standard amount will be applied by each of the 13(?) test subjects. So this entire Section 9.1.1 should be struck. In any case, we believe the calculations done here are incorrect and make a number of incorrect assumptions. More specifically, these include the assumption that the distribution of applied product is normally distributed. Given that the mean and SD are about equal (mean of 3.8 and SD of 3 or 3.5), this is not possible and permits doses to be negative. Further, our QQ plot of the data suggests these are not normally distributed, and that a log-normal distribution is much more reasonable. And thirdly, the calculations done below or the source of this calculation in the table are not clearly laid out. Since these include **replicate** samples, any standard sample size calculation using standard formulae would not be applicable (even ignoring the normal assumption that might be typical of closed-form calculations)

8.6. Follow-up after testing

Participants will be followed up within 72 hours after the test to assess any possible adverse events.

9. Statistics and Data Analysis

9.1. Sample size calculation

9.1.1. Dose determination

It is reasonable to assume that the CPT data of this product follows a Weibull distribution. Given the results of EPA simulation (Appendix 5) and the belief that P5MR (an expression of EPA for the variation of CPT data = 5th percentile/50th percentile) of the testing product is 0.5 or greater, a sample size of 13 subjects for this study design would provide sufficient power (>0.80) to obtain a ratio of the lower limit of 95% CI of the estimated median CPT /

estimated median CPT is ≥ 0.6 , where the ratio of the lower limit of 95% CI of the estimated median CPT/estimated median CPT expresses the precision of estimated median CPT.

Based on preliminary data with a mean of 3.8 and an SD of 3.5, we calculated the sampling error (confidence limit with) for a 95% confidence interval.

-	-	-
Volunteer 1	Replicates 1, 2, 3	0.52, 0.25, 0.43
-	Mean	0.40
-	-	-
Volunteer 2	Replicates 1, 2, 3	13.31, 5.33, 3.23
-	Mean	7.29
-	-	-
Volunteer 3	Replicates 1, 2, 3	3.49, 2.64, 3.49
-	Mean	3.20
-	-	-

The estimation of SD of 3.5 is inflated due to an extreme observation where the participant continued to apply product as the gauze prevented them from feeling as though enough had been applied, so, we expect that a value of 3 is more realistic.

The power calculation below indicates that for a 70% power, with a sample of 11 individuals it is possible to obtain a confidence interval of ± 1.5 units from the mean under the realistic scenario.

SD	SE	70%	80%
3	1.5	11	13
	2	7	9
3.5	1.5	12	15
	2	8	9

9.1.2 Complete protection time

Based on data from a previously completed field trial of mosquito repellents in Florida⁸, where the observed CPT ranged from 2.5 to 5.5 hours with a median of 5 hours, and where 1 out of 8 individuals was censored, a calculation of 95% confidence interval of the median was performed for this study based on a sample size of 10 individuals with the same parameters as above with 1 individual censored (i.e. 10%) at 5.5 hours. This obtained a confidence width of 3 hours. Regardless of the observed data the lower limit of this 95% confidence interval corresponded to the 3rd lowest observed time. Similar calculations were

Commented [NJ65]: The sample size and power calculation provided in this section is not clear. HED statisticians think the assumptions and logic made in this calculation may not be true for the data of this study. Therefore, HED statisticians conducted a simulation. This was discussed with the HSRB during a conference call with the HSRB during May 2017. Please Appendix 5 for more detail of the simulation. Since this has already been seen by the HSRB in the May Administrative conference call, it is probably best to cite this work and these simulations as the source of sample size number.

Note that what was sent to and reviewed by HSRB has been added as an Appendix to this protocol.

~~performed for the same sample size, but with 2 individuals censored (i.e. 20%), which resulted in identical results.~~

It is reasonable to assume that the CPT data of this product follows a Weibull distribution. Given the results of EPA simulation (Appendix 5) and the belief that P5MR (an expression of EPA for the variation of CPT data = 5th percentile/50th percentile) of the testing product is 0.5 or greater, a sample size of 13 subjects for this study design would provide sufficient power (>0.80) to obtain a ratio of the lower limit of 95% CI of the estimated median CPT / estimated median CPT is ≥ 0.6 , where the ratio of the lower limit of 95% CI of the estimated median CPT/estimated median CPT expresses the precision of estimated median CPT.

9.2. Data analysis

The endpoint for Complete Protection Time will be time to treatment failure for each participant test. Treatment failure is the time at which the product no longer provides complete protection, which is determined as the time at which one landing occurs in a ~~5 minute~~ 5-minute period, followed by a ~~confirmatory second~~ landing within the same 5-minute period or in the next 5 minute period (-30 minutes later). The times to treatment failure will be analyzed using Kaplan-Meier Survival functions, and from these the median Complete Protection Time and 95% confidence intervals will be calculated.

Adverse events will be tabulated and included in the study report. Adverse events occurring after the end of participant participation but before the end of the study will be listed separately.

10. Safety and Data Monitoring

10.1. Risk assessment

The Principal Investigator has determined studies of this kind to be "low-risk". Day-to-day monitoring will be carried out at the study center by a member of the study team with delegated responsibility. A separate monitoring team from *arctec* will monitor the study before participant enrolment, during the study and on study completion.

Safety information regarding the repellent used in the trial have been assessed, material and safety data sheets (MSDS) and labels have been read to be sure they are safe for human use. The active ingredient in the repellent to be tested is IR3535. Participants will be explained the details of the ingredients and what to do if they have a reaction to the product or the mosquitoes after completion of the test.

10.2. Adverse events

Volunteers will be monitored throughout the duration of the tests by investigational staff for any adverse events. Subjects will be told that if anyone experiences any skin reaction, experiences an injury, or simply feels unwell, he or she should inform the investigational staff right away. Such subjects will immediately be given appropriate care, may be withdrawn from testing, and may be transported to a local hospital if necessary. If any

Commented [BE66]: See comments above about two landings

Commented [FC67R66]: 2 landings within the 5-minute exposure period

Commented [AM68]: It seems that this discussion fits in the GLP/QA section suggested earlier. Please consolidate.

Commented [AM69]: Please include specific information toward the beginning of the protocol about IR3535 and the known risks associated with it.

Commented [AM70]: EPA recommends having at least one member of the investigational staff also trained in first aid, and/or having a nurse on call for the duration of the study.

adverse events related to insect exposure or the repellent product are apparent at any time during the trial, testing will stop immediately and details of how to access treatment and how treatment will be paid for will be offered. The likelihood of adverse effects occurring is very low because participants with known allergies to insect bites or any of the product ingredients will not be eligible to take part. An adverse reaction to an insect bite is defined as a weal greater than 1 cm in diameter that is very red or very itchy.

Commented [AM71]: Please clarify. How will the subject be transported for medical care? EPA recommends that a member of the study team accompany the subject experiencing an adverse reaction to the medical facility. Will testing for all subjects stop, or just the subject who is having an adverse reaction? What will happen with the subjects who may continue testing? How will their transportation be handled at the end of the testing period?

If requested by the subject, standard over-the-counter first aid items such as bandages, antiseptics, and hydrocortisone cream, will be provided immediately upon completion of the test at no cost to the subject. They may also request first aid assistance at any time. A nurse will be contacted prior to the test date and will be on call during each test day for non-emergency queries or problems. Within 72 hours after field testing an email will be sent to participants asking them to report any adverse events that might have occurred since the end of testing. Adverse events that occur >72 hours after the end of participation in the trial will be passively monitored.

Commented [AM72]: Please explain whether you will follow up if a subject doesn't respond to the 72 hour follow up email.

All adverse events should be recorded on the Adverse Event Record Form (Appendix 1) and Adverse Event Monitoring Questionnaire (Appendix 2) and entered into a spreadsheet and stored on a secure drive. All adverse events will be reported to the Sponsor. Depending on the nature of the event the reporting procedures below should be followed, see Appendix 3 for a flowchart of safety reporting.

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Commented [AM73]: Need to explain who will exercise medical judgment and how/when. Should be able to ID someone on staff/independent (e.g., contract nurse/first aid person) to do the evaluation according to specific criteria.

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the definitions in section 7.1. AE questionnaires meeting the SAE definition will be submitted to the Principal Investigator, Dr Emma Weeks within 24 hours. SAEs that are assessed by the Principal Investigator as being both related and unexpected must be reported to the UF IRB within 5 days of the Principal Investigator becoming aware of the event.

In the case of a severe reaction such as anaphylaxis, a trained First Aider will be called immediately and the volunteer taken to the nearest Emergency Room (ER).

Commented [AM74]: If risk of anaphylaxis is low/negligible, be explicit about the potential risk and note what precautions will be taken to provide medical assistance on site (e.g., trained first aid person/nurse, epi pens). If there is no or negligible risk of anaphylaxis during field testing, given the screening process and mosquito attractiveness test, then note that in the protocol.

An adverse event which is ongoing at the time of participant withdrawal or completion will be followed up until it resolves or until 30 days after the participant terminates from the study, whichever comes first.

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or study participant
Serious Adverse Event (SAE)	<p>A serious event is any untoward medical occurrence that:</p> <ul style="list-style-type: none"> Results in death Is life-threatening Requires inpatient hospitalization or prolongation of existing hospitalization Results in persistent or significant disability/incapacity Consists of a congenital anomaly or birth defect <p>Other 'important medical events' may also be considered serious if they jeopardize the participant or require an intervention to prevent one of the above consequences.</p>

10.3. Compensation due to adverse events

If ~~you-a subject is/are~~ injured as a direct result of ~~your-his or her~~ participation in this study, the Sponsor will pay for all reasonable and necessary medical expenses required to treat ~~your-the~~ injury, as long as:

1. The injury occurs during ~~your-the subject's~~ participation in the study.
2. The injury results directly from the study product or study-required procedures.

The Sponsor and the Principal Investigator will determine whether ~~your-the~~ injury is related to ~~your-the subject's~~ participation in this study. To do this, they may request to consult with the person/facility that provided medical treatment following an adverse effect, which could require your consent.

No additional compensation for adverse events beyond payment for medical expenses related to participation in the study is routinely offered. The Principal Investigator and others involved in this study may be University of Florida employees. As employees of the University, they are protected under state law, which limits financial recovery for negligence.

Please contact the Principal Investigator Dr. Emma Weeks on 352-870-4327 if you experience an injury or have questions about any discomforts that you experience while participating in this study.

10.34. Data monitoring

With the exception of the Volunteer Questionnaire, which is completed by the participant, all data collected will be recorded in the case report form (CRF) and signed by the person completing the CRF. The CRF is considered to be source data.

Data to be collected are: Participant number and date of visit on every page, confirmation of informed consent, date of birth, eligibility details, mosquito attraction details, test visits (eligibility checklist, product details, product application details, field testing data (time, fitness check, No. insects landing) and adverse event monitoring.

Raw data from the CRF are then entered into an Excel spreadsheet for analysis and saved on a secure drive. Data will be double entered and verified to ensure accuracy. CRFs will be kept in locked storage.

Information in the database for each test will be linked to a relevant SOP, risk assessment, contract, and files of statistical analysis.

11. Regulatory Issues

11.1. Ethics approval

The PI at the Entomology and Nematology Department (University of Florida, Gainesville, FL, USA) will obtain ethical approval from the UF Institutional Review Board (IRB).

11.2. Consent

Consent to enter the study must be sought from each volunteer only after a full explanation has been given, and time allowed for consideration. Signed volunteer consent will be obtained. The right of the participant to refuse to participate and to withdraw at any time without giving reasons must be respected.

11.3. Confidentiality

Participants' identification data will be required for the enrolment process. The Trial Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

The information obtained from subjects taking part in these studies will be used by the researchers, funders, and the sponsor, and will become part of a series of reports (one report for each conducted study). All reports (as well as all study-related records) and will be kept as confidential as possible under local, state, and federal laws. The results of this study are not intended for publication; however, if any of the study-related data are published, subjects' identities will remain confidential.

All efforts will be taken to maintain the confidentiality of the pregnancy test results. The test results will not be recorded, and will not be disclosed to anyone other than the test subject, the verifying employee, and/or the Study Director. Opaque bags will be available where the pregnancy tests are taken to allow for discrete disposal.

In addition, the subjects' identities will be protected as follows: each subject will be assigned a code number, and only subjects' code numbers will appear on data sheets. The subjects' names will not appear anywhere on the data sheet, or in the reports. The study records will be maintained at the testing facility in locked cabinets and electronic files kept on a password-protected computer server. No one outside researchers, Sponsor, the IRB, or certain governmental agencies (such as USEPA) will have access to subjects' personal information.

11.3.1 How will health information be collected, used and shared?

The Principal Investigator will create, collect, and use protected health information or PHI. This information can be gathered from the participants and any tests explained in previous sections of this protocol. This information will be obtained during study visits and telephone calls.

More specifically, the following information may be collected, used, and shared with others:

- Name will be collected and assigned a coded subject identifier based on gender, such as Subject M1, F2, F3, M4, etc.
- Age will be requested because participation of those under the age of 18 or over the age of 55 is prohibited for this study.
- Gender may be reported with the data as the coded identifier above, where M is for male and F is for female.
- Information about health including: general health, allergies, pregnancy (if applicable), insect bite history, and participation in other studies.
- ~~phone~~ Phone number and email address
- ~~social~~ Social security numbers: for compensation payments

This information will be stored in locked filing cabinets or on computer servers with secure passwords, or encrypted electronic storage devices.

If limited data sets are created and used, agreements between the parties creating and receiving the limited data set will be obtained.

11.3.2. For what study-related purposes will PHI be collected, used, and shared with others?

PHI may be collected, used, and shared with others to make sure participants are eligible to take part in the research, through participation in the research, and to evaluate the results of the research study.

More specifically, PHI may be collected, used, and shared with others for the following study-related purpose(s):

- The purpose of this study is to evaluate the repellency of novel and commercial repellents against mosquitoes that bite humans and other animals. Some of the results from these studies will be averaged and reported and published in scientific journals.
- Some results from these studies may be kept confidential so that only the PI, their staff and the repellent manufacturer are aware of the results.
- Once this information is collected, it becomes part of the research record for this study.

11.3.3. Who will be allowed to collect, use, and share PHI?

Only certain people have the legal right to collect, use and share the research records, and they will protect the privacy and security of these records to the extent the law allows. These people include:

- the study Principal Investigator (listed in question 3 of this form) and research staff associated with this project.
- other professionals at the University of Florida or Shands Hospital that provide study-related treatment or procedures.
- the University of Florida Institutional Review Board (IRB; an IRB is a group of people who are responsible for looking after the rights and welfare of people taking part in research).

11.3.4. Once collected or used, with whom may PHI be shared with?

PHI may be shared with:

- the study Sponsor (listed in Part 4 of this form).
- United States agencies who are responsible for overseeing research, such as the Food and Drug Administration, the Department of Health and Human Services, the Environmental Protection Agency, and the Office of Human Research Protections.

- Government agencies who are responsible for overseeing public health concerns such as the Centers for Disease Control and federal, state and local health departments.

Otherwise, research records will not be released without permission unless required by law or a court order. It is possible that once this information is shared with authorized persons, it could be shared by the persons or agencies who receive it and it would no longer be protected by the federal medical privacy law.

11.3.5. How long will PHI be used and shared with others?

PHI will be used and shared with others until the end of the study. If the consent form is signed then the participant is authorizing researchers to collect, use and share PHI. Participation is not permitted in this research unless you allow the collection, use and sharing of your protected health information by signing this consent and authorization.

Participants can revoke authorization at any time before, during, or after your participation in this study, by giving a written request with a signature on it to the Principal Investigator. If authorization is revoked, no new information will be collected. However, information that was already collected may still be used and shared with others if the researchers have relied on it to complete the research.

11.4. Sponsor

LivFul Inc. is the Sponsor. Address: 2972 Webb Bridge Rd, Alpharetta, GA 30009, USA

11.5. Funding

LivFul Inc. will fund the study. Participants will be offered up to ~~\$120-200~~ per test (~~\$10 per hour for the first 8 hours, \$15 per hour for any hours beyond the first 8, rounded up to the next hour~~); 6 visits in total) and ~~\$20 per meeting (up to \$60)~~ for attending the consenting, enrollment and training visits, to defray the cost of attending. If they test one product at one site, this is a total of ~~\$180~~260. If they test all three products at both sites (6 complete tests) this is a total of ~~\$780~~1260.

Commented [AM75]: How will alternates be compensated if they are present on test days but not needed to participate? How will people who begin a test but decide to withdraw be compensated? How will people who do not comply with instructions and are asked to withdraw be treated?

See sample language below.

Commented [AM76]: EPA suggests offering time and a half for hours beyond 8 hours/full day of work, so payment for a 12-hour day would be \$10/hour for 8 hours and \$15/hour for anything over 8 hours.

Visit	Reason			Compensation
1	Consenting			\$20
2	Enrollment and Dose Determination			\$20
3	Mosquito attractiveness and training			\$20
4	Site 1	Product 1	Test 1 (1-4 days)	\$120 <u>200</u>
5	Site 1	Product 2	Test 2 (1-4 days)	\$120 <u>200</u>

Commented [AM77]: With the elimination of the dose determination phase of the study, will there be 2 meetings, or will the mosquito attractiveness test and aspirator training occur together?

6	Site 1	Product 3	Test 3 (1-4 days)	\$120 200
7	Site 2	Product 1	Test 4 (1-4 days)	\$120 200
8	Site 2	Product 2	Test 5 (1-4 days)	\$120 200
9	Site 2	Product 3	Test 6 (1-4 days)	\$120 200
Total				\$7801260

Each subject will be paid \$20 for taking part in each training session.

For each test day, test subjects will be paid \$80.00 (\$10 per hour) for any length of participation up to 8 hours. If a test day exceeds 8 hours, subjects will be paid \$15/hour (time and a half) for each additional hour, rounded up to the nearest hour.

An alternate who is not needed to replace a test subject will be able to leave and will be paid \$TBD. The decision as to whether an alternate is needed will occur within the first two hours of the test, before all the treatments have been finished. If an alternate is asked to replace a subject, he or she will be paid at the same rate as other test subjects, as described above.

Subjects who have participated in the training session, but then choose to withdraw or are asked to withdraw from or during the training session, will still be paid \$20 for attending all or part of this session.

Subjects may decline to participate at any time during the training session or test day without penalty. Subjects will be compensated for their time up until their decision to withdraw.

If the Study Director or other study staff ask a subject to withdraw from the test and they have complied with all of their requests, or if a test subject needs to withdraw early because of a health or emergency reason, full payment will still be made even if the test subject has participated for less than eight hours. This will not affect payment for any previous test days that the subject has completed.

The Study Director or other designated investigational staff may end a particular subject's participation in a training session or on a test day, at any time, for any reason. If a test subject is asked to withdraw from the test because they have not followed directions from the investigational staff or if they choose to withdraw from testing early on a test day for a non-health related or non-emergency reason, they will be paid for the number of hours worked (rounded to the nearest hour) at a rate of \$10.00 per hour for the first 8 hours, and \$15 per hour for any hours beyond the first 8 hours. This will not affect payment for any previous test days that had been completed.

Commented [AM78]: Tailor this to address payment for consent, attractiveness test, and training

Subjects will be paid on (e.g., 1st and 15th of the month) by (mail, wire transfer, check delivered at the office, etc).

Commented [AM79]: Include information on how/when subjects will be paid.

11.6. Record retention

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

12. References

~~1. Safety Profile Insect Repellent IR3535® – EMD Performance Materials, 2016.~~

2. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996) 'ICH Harmonised Tripartite Guideline E6(R1): Guideline for Good Clinical Practice'.

3. World Medical Association Declaration of Helsinki (1964) 'Ethical Principles for Medical Research Involving Human Subjects'.

4. Data Protection Act 1998.

Commented [AM80]: Please reference most updated Declaration of Helsinki (October 19, 2013)

<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>

Commented [AM81]: Cite please – what country, provide URL

5. Smartt, CT, TMS Stenn, T Chen, MG Teixeira, EP Queiroz, LSD Santos, GAN Queiroz, KR Souza, LK Silva, D Shin & WJ Tabachnick. 2017. Evidence of Zika virus RNA fragments in *Aedes albopictus* (Diptera: Culicidae) field collected eggs from Camaçari, Bahia, Brazil. J Med Entomol (ACCEPTED).

6. Smartt, C.T., Shin, D., Anderson, S.L. 2016. The Effect of West Nile Virus Infection on the Midgut Gene Expression of *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae). Insects 7, 76; doi:10.3390/insects7040076

7. Merck. The Effectiveness of IR3535® Against Mosquitoes.

http://us.ir3535.com/en/effectiveness_of_ir3535/against_mosquitoes/against_mosquitoes.html

8. EPA (2016). Science Assessment: Field Testing of S.C. Johnson Personal Mosquito Repellent Mark-4 Product to Support the Use of the EPA Repellency Awareness Graphic https://www.epa.gov/sites/production/files/2016-11/documents/final_mark_4_study_science_and_ethics_presentation.pdf

Appendix 1. Adverse Event Record Form

Study Title:

Volunteer reference number	Date	Side effect/adverse event	Related to the product	Comments	Action taken
			Y/N/ don't know		

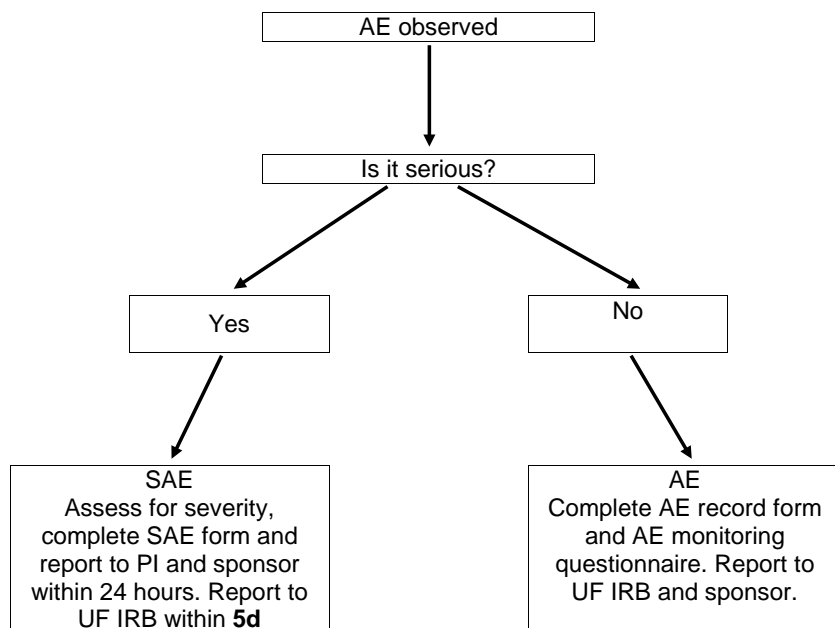
Appendix 2: Adverse Event Monitoring Questionnaire

To be completed by person who experienced the Adverse Event	
Name (first name SURNAME)	
Date of birth dd/mm/yyyy	
Phone number	
Mobile number	
E-mail address	
What kind of adverse event did you experience? e.g. skin rash, burning sensation, severe allergic reaction	
How long did the adverse event last?	
How serious was the event? mild / moderate / severe / life threatening	
Did you take any action to resolve the event?	
Was any treatment required?	
Did you visit A&E? Please enter details	
Did you stay in hospital overnight? Please enter details (no. nights/admission)	
Outcome unresolved / resolved / resolved with sequel	
To be completed by trial manager	
Participant ID	
Study title	
Study code	
Type of study e.g. repellent, impregnated clothing, after-bite cream	
Exposure type e.g. chemical, mosquito bites, bed bug bites	
Exposure area e.g. forearm, legs, hands	
Active ingredient	
Report date dd/mm/yyyy	
Other event Please enter details	

Likelihood of Adverse Event being related to study unrelated / unlikely / possible / probably / definite	
Serious Adverse Events	
Was the event serious? yes / no	
Admitted to Intensive Care Unit? yes / no	
Seriousness criteria (please tick)	
life threatening	
required hospitalisation	
prolonged hospitalisation	
congenital anomaly	
disabling/incapacitating	
important medical event	
required intervention to prevent impairment or damage	
Fatal	
If fatal, date of death dd/mm/yyyy	
Primary cause of death	
Was a post-mortem performed? yes / no	
Date adverse event become serious dd/mm/yyyy	
Possible contributing factors to SAE other than study participation or underlying disease being studied Please give details	
None apparent	
Concurrent illness, disease or other external factors	
Concurrent medication	
Study procedure	
Accident, trauma, or other external factors	
Other	
Relevant concomitant medication at time of SAE yes / no – if yes please provide details	
Treatments/procedures for SAE yes / no – if yes please provide details	

Relevant medical history (include only relevant past or concurrent medical disorder, surgeries, etc that might help explain the SAE) yes / no – if yes please provide details	
Relevant laboratory testing yes / no – if yes please provide details	
If relationship to study participation was unrelated, provide causality Please give specific details	
Discontinuation of study participation	
Concurrent disorder	
Concomitant medications	
Other	
If action taken with study participation, was study interrupted or discontinued? Provide date (dd/mm/yyyy)	
Did SAE abate after study was stopped? yes / no / not applicable / unknown	
Did SAE reoccur after reintroduction of study participation? yes / no / not applicable / unknown	
Narrative/Comments Please describe the SAE including a chronological clinical presentation and evolution of the SAE and associated signs/symptoms	
Please submit this questionnaire to Dr Emma Weeks. SAEs that are assessed by the PI as being both related and unexpected must be reported to the UF IRB within 5 days of the PI becoming aware of the event.	

Appendix 3: Flowchart for Safety Reporting



Appendix 4: Calculations of Maximum Safe Dosage of IR3535

Participant	AEL* (mg/kg bw/day)	Weight* (kg)	Max Internal Dose IR3535@ (mg/day)	Dermal Absorption* (%)	Max External Dose IR3535@ (mg/day)
Adult Male	5	73.80	369	14	2635.71
Adult Female	5	60	300	14	2142.86

* Accepted exposure level (AEL) and Dermal Absorption taken from: Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products: Evaluation of active substances Assessment Report: Ethyl butylacetylaminopropionate Product type 19 (insect repellent) (2013) (CA-Dec13-Coc.3.4a—IR3535 draftAR.docx).

** Weight taken from: U.S. Environmental Protection Agency (EPA). (2011) Exposure Factors Handbook: 2011 Edition. National Center for Environmental Assessment, Washington, DC; EPA/600/R-09/052F.

Participant	Max External Dose IR3535@ (mg/d)	Product: -% IR3535@	Max External Dose Product (mg/d)	Lower Leg Surface Area (cm ²)	Product Application Rate (mg/cm ²)	Amount of Product Applied to Lower Leg (mg/cm ²)	Amount of Product Applied as-% of Max External Dose (IR3535@-or Product)
Adult Male	2635.71	15%	17571.43	1044	1.67	1743.48	9.92
Adult Male	2635.71	17%	15504.20	1044	1.67	1743.48	11.25
Adult Male	2635.71	20%	13178.57	1044	1.67	1743.48	13.23

Appendix 4 – Basis for Standard Dose

In place of the dosimetry phase, EPA recommends a standard dose of 1 g product per 600 cm² skin area for all products except pump sprays. For pump spray products, the Agency recommends 0.5 g product per 600 cm² of skin area.

According to the EPA's risk assessment based on data submitted to EPA for registration of IR3535, IR3535 is not a skin sensitizer, is classed as category III for acute dermal toxicity (LD₅₀ > 3000 mg/kg in rats), category IV for acute oral and inhalation toxicity (LD₅₀ > 5000 mg/kg in rats), and category II for eye irritation. The NOAEL for dermal toxicity is ≥ 3000 mg/kg/day in rats and for oral toxicity is 600 mg/kg/day in rabbits. In its risk assessment, EPA used a 5% dermal absorption factor for IR3535 and based their calculations on an 11.8 kg child and 60 kg adult for a product at 7.5% active ingredient.

The calculation below is an example for calculating exposure estimates for a product containing 20% IR3535, the worst case scenario of the three products proposed in the protocol, for risk characterizations using 2680 cm² as the average area for the lower leg of an adult male (average lower leg area for a female is 2330 cm²):

$$(2680 \text{ cm}^2 \times 1000 \text{ mg formulation}/600 \text{ cm}^2) \div 60 \text{ kg} = 74.4 \text{ mg formulation/kg}$$

$$(74.4 \text{ mg formulation/kg})(0.20 \text{ mg a.i./mg formulation}) = 14.9 \text{ mg a.i./kg}$$

$$14.9 \text{ mg a.i./kg} \times 0.05 \text{ dermal absorption factor} = 0.75 \text{ mg a.i./kg}$$

Margins of Exposure (MOE) are calculated by dividing the oral NOEL of 600 mg/kg/day as below:

$$600 \text{ mg/kg} \div 0.75 \text{ mg/kg} = 800$$

To reach the EPA's level of concern (MOE ≤ 100), a person would need to make approximately 9 applications in a single day.

In conclusion, the amount of IR3535 applied in these studies does not exceed a level of toxicological concern to test subjects.

Commented [FC82]: Information in Appendix 4 will be replaced with calculations from Eric's review in review memo, supported by Executive Summary document on toxicity assessment for IR3535

Appendix 5: Power/Sample Size Calculation

Objective

To determine the sample size N such that mosquito repellency studies have sufficient power to obtain a given degree of **precision** in the estimate of median Complete Protection Time (mCPT). This precision – designated as “ K ” – will be expressed as the ratio: $95\% \text{ LCL}_{\text{mCPT}}/\text{estimated mCPT}$

The simulation used to estimate varying sample sizes will require that that $95\% \text{ LCL}_{\text{mCPT}}/\text{estimated mCPT} < K$; true **variation** of the Complete Protection Time (CPT) distribution will be expressed by the Weibull distribution family and a parameter, $P5MR$, defined as the 5th percentile/mCPT.

In order to develop estimates of a required sample size for a mosquito repellency study to achieve certain stated efficacy criteria and estimate a complete protection time (CPT)¹, it is necessary to determine the distribution of mosquito repellent failure times (generally considered to be time to first landing with intent to bite). However, the underlying distribution of the CPT of a product being tested in a mosquito repellency study is not known prior to the testing phase. What is known about the distribution is that CPT values are (necessarily) non-negative and are (generally) right censored after 10 (or 12 hours) in most mosquito repellency studies.

On this basis, EPA assumed for this sample size determination exercise that a distribution of mosquito repellent failure times follows a Weibull distribution. A Weibull distribution is commonly used in reliability engineering and failure analysis, in survival analysis, in predicting delivery times, in weather forecasting and hydrology, and in extreme value prediction. Its utility in a wide variety of applications is due in part to its flexibility to take on a variety of shapes depending on the parameters selected to describe the distribution. Oftentimes, the Weibull plot is described by two parameters: κ (the “shape” parameter and sometimes referred to in some parameterizations as “ a ”) and λ (the scale parameter and sometimes referred to as “ b ”).² The PDF (probability density function) and CDF (cumulative distribution function) of the aforementioned two-parameter Weibull distribution are defined, respectively, as follows:

$$f(x, \kappa, \lambda) = \begin{cases} \frac{\kappa}{\lambda} \left(\frac{x}{\lambda}\right)^{\kappa-1} e^{-(x/\lambda)^\kappa} & x \geq 0, \\ 0 & x < 0 \end{cases}$$

$$F(x, \kappa, \lambda) = \begin{cases} 1 - e^{-(x/\lambda)^\kappa} & x \geq 0, \\ 0 & x < 0 \end{cases}$$

and are illustrated in the associated plots in Figures 1 and 2 for some illustrative κ and λ values.

Parameterizing the Weibull distribution in terms of κ and λ is, however, not necessarily intuitive with respect to studying – and judging -- the efficacy of skin-applied mosquito repellents as measured by CPT for individuals using the repellent in the field. Instead, it is more natural and desirable to be able to express the efficacy of the repellent in terms of both the expected precision of the estimated median CPT (mCPT) and in terms of the estimated variability of mCPT in (or across) the population. More specifically: the testing of a given repellent in the field should be able to generate a reasonably precise estimate of the mCPT that is expected to be generally close to what a sizable fraction of the population

¹ The Complete Protection Time (CPT) is defined as the time from initial application of the repellent by the test subject to the time of first confirmed landing with intent to bite (FCLIB). The FCLIB is considered to be when one landing is followed by another landing within 30 minutes. The first landing is confirmed by the second landing.

² A Weibull distribution can sometimes be described by 3 parameters, with a “location” parameter added as a third parameter to the “scale” and “shape” parameter of the 2-parameter Weibull distribution.

would be expected to experience (or, more accurately, a mCPT that only a small fraction of the population would ideally experience to be much shorter).

Following the above logic, we define the *precision of the CPT estimate* -- designated as "K" -- as follows:

$$K = 95\% \text{ LCL}_{\text{mCPT}} / \text{estimated mCPT}$$

where: mCPT = median complete protection time

95% LCL_{mCPT} = 95% lower confidence limit on the estimated mCPT

Similarly, the degree of variation of the CPT distribution in the population will be defined as the P5MR which we define here as the ratio between the mCPT of the 5th percentile of the population to the mCPT of the population:

$$P5MR = \text{CPT}_{5\text{th \%ile}} / \text{mCPT}$$

where:

mCPT = median complete protection time

CPT_{5th %ile} = 5th percentile of the distribution of CPT

Re-parameterization of Standard Weibull Equation

While the above mCPT and P5MR parameterizations of the Weibull distribution are intuitively appealing for judging and evaluating repellent efficacy, they are non-standard parameterizations and it is necessary -- for comparison and simulation purposes -- to convert these to the more standard κ (shape) and λ (scale) values. To do this, EPA developed an equation such that interconversion between the standard (κ (shape) and λ (scale)) parameterization of the Weibull to this alternate version (with the Weibull distribution instead expressed in terms of P5MR and mCPT). Briefly, the cumulative probability function of CPT is assumed to be a 2- parameter Weibull distribution:

$$P(\text{CPT}, \kappa, \lambda) = 1 - e^{-(\text{CPT}/\lambda)^\kappa}$$

Given that a value of the mCPT represents the median or 50th percentile of the CPT and the value of P5MR represents the ratio of the 5%-tile of the CPT distribution to the mCPT, we can develop the following two equations to represent the cumulative distribution functions at the median CPT and the 5th percentile CPT:

$$P(\text{mCPT}, \kappa, \lambda) = 1 - e^{-\left(\frac{\text{mCPT}}{\lambda}\right)^\kappa} = 0.5 \quad (\text{median})$$

$$P(P5MR \times \text{mCPT}, \kappa, \lambda) = 1 - e^{-\left(\frac{P5MR \times \text{mCPT}}{\lambda}\right)^\kappa} = 0.05 \quad (5\text{th percentile})$$

Algebraically solving the equations above (see Appendix 5A for full derivation), we develop expressions for κ and λ :

$$\kappa = \frac{\ln\left[\frac{\ln(0.95)}{\ln(0.5)}\right]}{\ln(P5MR)}$$

$$\lambda = \frac{1}{e^{\frac{1}{\kappa}}} \times \ln\left[\frac{\text{mCPT}^\kappa}{\ln(0.5)}\right]$$

Table 1 below compares these two parameterizations for the example PDF and CDF distributions shown in Figures 1 and 2, respectively, for the κ and λ parameterizations shown there, illustrating the conversion to this new parameterization:

Table 1. Re-parameterization of Weibull Distribution Parameters from Traditional (κ, λ) to Revised (P5MR, mCPT) for Example Weibull Distributions Appearing in Figures 1 and 2.				
Parameterization Scheme				Description/Comments
Traditional		Revised		
Scale (λ) ^a	Shape (κ)	mCPT ^b	P5MR ^{c,d}	
1	0.5	0.480453	0.005476	- κ values of less than 1 indicate a failure rate decreases over time, and defective items fail early or are otherwise removed from the population.
1	1	0.693147	0.074001	- κ values equal to 1 indicate a constant failure rate over time possibly suggesting failure is due to random external events. - Here, the Weibull distribution reduces to the “exponential” distribution; - Note that mCPT here = 0.693 = ln(2)
1	1.5	0.78322	0.176261	- κ values greater than 1 suggests that the failure rate increases over time, as when there is an “aging” process or components are more likely to fail over time.
1	5	0.92932	0.594083	

^aThe Weibull scale parameter is the 63.2 percentile of the distribution. If the scale parameter is 1, then this means that 63.2% of the observed values will be smaller than 1. Note in the CDF in Figure 2, as a consequence, that all $\lambda=1$ distributions intersect at the 63.2 percentile.

^b $mCPT = \lceil \ln(2)^{\kappa} \exp(\kappa \cdot \ln(\lambda)) \rceil^{1/\kappa}$

^c $P5MR = \exp(\ln(\ln(0.95)/\ln(0.5))/\kappa)$

^dNote that as κ increases, the P5MR value becomes larger, indicating that the values at the 5th percentile approaches the values present at the 50th percentile, and the PDF becomes tighter and more peaked. κ values of between 3 and 4 often lead to distributions that appear normal.

An example of the (varied) kinds of distributional “shapes” associated with various parameterizations is shown in Figure 2 as histograms of the CPT. More specifically, Figure 2 presents the CPT distributions with different medians and values of P5MR (ratio 5%-tile/mCPT). These present the CPT distributions with different mCPTs (2-, 4-, 6-, and 8- hrs) and values of the P5MR ratio (P5MR= 0.2, 0.3, 0.4, and 0.6) for the (assumed) Weibull Distribution³. As seen in Figure 2, larger mCPTs are associated with a shift in the distribution toward the right. In addition -- and importantly -- smaller P5MR values in this range are associated with “flatter” distributions and larger P5MRs are associated with more “peaked” distributions, with these more peaked distributions showing a greater percentage of the distribution centered around the median. From a regulatory perspective, a CPT distribution with a larger P5MR is more desirable than a CPT distribution with smaller P5MR since this means that a greater percentage of the user population experiences an actual CPT closer to the (advertised) mCPT. Further, it could be argued from a

³ Other simulations were performed for the lognormal, normal, and uniform distributions, with the latter one (particularly) done as a form of sensitivity analysis but these are not discussed in this report; the simulation outputs, however, are provided in Appendix 5D. Note that the power estimates for a given sample size from the Weibull and Lognormal distributions are similar.

public policy perspective that a large variability in CPT in the population for a given repellent is not a desirable characteristic, and does not accurately portray or indicate any “expected” mCPT on the part of the consumer.

OPP staff have judged what might be considered reasonable values for input parameters (precision of the estimated mCPT and variability in CPT in (or among) users of the tested product) in order to estimate required number of test subjects for the field exercise to achieve a desired set of aims regarding precision around the estimate of the mCPT. These judgments are based in part on available data and past experiences⁴ and in part on general thoughts regarding consumer expectations with respect to product efficacy. Specifically, EPA has estimated the power associated with various sample sizes where power – as defined here – is the probability that the ratio of the (95% LCL_{mCPT})/(estimated mCPT) is greater than a given acceptable K (a scalar which measures the precision of the estimates in estimating the mCPT). Such mosquito repellency study design power depends on:

- Number of test subjects
 - The larger the number of test subjects, the greater the power
- (The required) precision (K) for estimated mCPT
 - The precision of an estimated mCPT from a study is expressed by the value of the ratio 95% LCL_{mCPT}/estimated mCPT. The value of ratio is in the interval (0, 1).
 - K is the smallest acceptable value of the ratio 95% LCL_{mCPT}/estimated mCPT for a given trial to be considered a “success”, and conceptually represents an inverse of precision (“tightness”) in the estimate of the mCPT: a larger K represents a greater “tightness” around the estimated mCPT. As K is chosen to be smaller, there is a greater probability that ratio 95% LCL_{mCPT}/estimated mCPT > K (and the trial is considered to be a “success” in the power calculation)
- P5MR
 - P5MR = ratio of the 5th percentile/mCPT
 - As the variation (dispersion or spread) of the distribution of CPT in the population becomes smaller, the 95% confidence interval of the estimated mCPT also becomes narrower (i.e. the 95% LCL_{mCPT} is closer to the estimated mCPT and the mCPT is better estimated, *certeris paribus*). Therefore, a smaller variation in the distribution of CPT will result in a larger P5MR and a higher probability that the ratio 95% LCL_{mCPT}/estimated mCPT > K. A CPT distribution with greater P5MR is generally more desirable than a CPT distribution with smaller P5MR

Ideally, a mosquito repellency study will be designed to have a sufficient number of test subjects such that one can have reasonable assurance that there is adequate power (defined here as a high probability that the ratio 95% LCL/estimated mCPT > K) given a shape and spread of the CPT distribution in the population. This shape/spread of the CPT in the population is defined by the P5MR.

⁴ See Appendix 5B for Weibull parameters fit to previous mosquito efficacy field data that the EPA has evaluated for a similar design and experimental set-up. In general, the values found in these (prior) studies support the values selected here to be used for the simulation

Brief Description of the Conduct of a Field Mosquitoes Repellent Study

In mosquito field repellency studies, test subjects are exposed in the field for 5-minute intervals immediately following product application and then for 5 minutes every 30 minutes until a “first confirmed landing” occurs. For subjects who receive confirmed landings, the CPTs are set as 0 if the first confirmed landing occurs during the first 5 minutes after application of the repellent; otherwise, the CPTs are rounded down to the nearest half hour (i.e., the starting time of the exposure period in which the first confirmed landing occurs). For those subjects for which there are no confirmed landings through the end of the testing day, CPTs are considered to be right censored at a time that is rounded down to the nearest half hour.

Description of (Computer) Simulation Procedure:

To simulate the field study trials, 4000 datasets were created with each dataset consisting of 10 data points (representing CPTs of 10 subjects) that were generated randomly from a Weibull distribution with a median CPT=2 and ratio of the 5%-tile/median P5MR= 0.2. If the randomly generated CPTs for the 10 subjects are $\leq 5, 6-35, 36-65, 66-95, \dots 576-605$ minutes, the CPTs are set to be 0-, 0.5-, 1-, 1.5- hours...10 hours, respectively, to simulate the study design in which each study participant is exposed for 5 of every 30 minutes until the first confirmed mosquito landing. If the randomly generated CPTs are greater than 10 hours (or 605 minutes), they are considered in the calculation to be (right) censored at 10 hours.

After generating the CPTs as described in the previous paragraph, the Kaplan Meier Estimator is used to estimate the mCPT and its 95% CI for each of the 4000 (10-person) datasets. The proportion of datasets in which the ratio of 95% LCL_{mCPT}/mCPT $> K$ as 0.6 is considered to be the “power” of the study design. More specifically: if the value of 95% LCL/mCPT > 0.6 is considered a “success”, the power is calculated as the proportion of successes in the 4000 datasets consisting of 10 data points each.

The process described in previous paragraph is then repeated for each combination of different mCPT = 2, 4, 6, and 8 hours; P5MR = 0.2, 0.4, 0.5, 0.6, 0.7, and 0.8; sample size per dataset = 10, 11, 12 ... 20; and the lowest acceptable K = 0.6, 0.7, and 0.8; all assuming that CPT follows a Weibull distribution⁵.

Results of Simulation

⁵ Such calculations were similarly done for the lognormal distribution, normal distribution, and uniform distribution, but are not discussed further in this report. The SAS output from these calculations and various associated tables and graphs, however, is shown in Appendix 5D for completeness.

Tables 1, 2, and 3 present the power estimates from simulations in which the data were randomly generated from Weibull distributions for $K = 0.6, 0.7$, and 0.8 , respectively. These are shown for various values of mCPT (ranging from 2 to 8 hours), P5MR (ranging from 0.2 to 0.8), and Sample Size (ranging from 10 to 20). As described earlier, K reflects a measure the precision of the estimate of mCPT with larger K values representing tighter estimates. For example, the K value of 0.6 requires that the 95% LCL on a median protection of 10 hours be no less than 6 hours (for a “success”) while a K value of 0.8 requires that the 95% LCL on that same median protection time be no less than 8 hours. A required precision of a K of 0.8, then, requires a more precise estimate of the mCPT than a K of 0.6 for this trial to be considered a “success” in the power calculation.

Figures 4, 5, and 6 present visually the same results in Tables 1, 2, and 3 (as power curves rather than tables).

As can be seen within each Table or Figure, the power of a study to achieve a given acceptable ratio K value (e.g., 0.6, 0.7, or 0.8 representing 95% $LCL_{mCPT}/mCPT$) value increases as the assumed P5MR value of the distribution increases (for example, from 0.2 to 0.8) or as the sample size increases (from 10 to 20). This is expected since a tighter (or more “peaked”) distributions (as evidenced by a larger P5MR value) will require fewer random “draws” to accurately estimate the mCPT. Across the Figures or Tables, we also see that as the acceptable K value increases from 0.6 to 0.8, the power of a study to achieve “95% $LCL_{mCPT}/mCPT > K$ ” decreases since stricter requirements for a “success” are being levied.

The SAS Code used to generate the simulated data and the associated tables and graphs are presented in Appendix 5C. Note - as described earlier - that simulations were also performed for the lognormal, normal, and uniform distributions, in part to serve as a sensitivity analysis and these are presented in the Appendix 5D for completeness, but are not discussed further here.

Figure 1. Probability Density Function (PDF) for Weibull Plot with λ (scale) =1 and κ (shape) ranging from 0.5 to 5

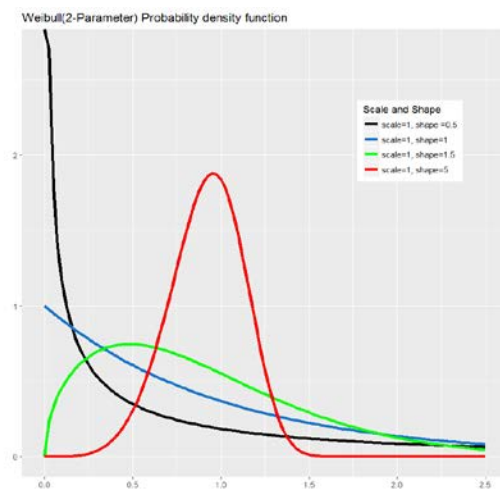
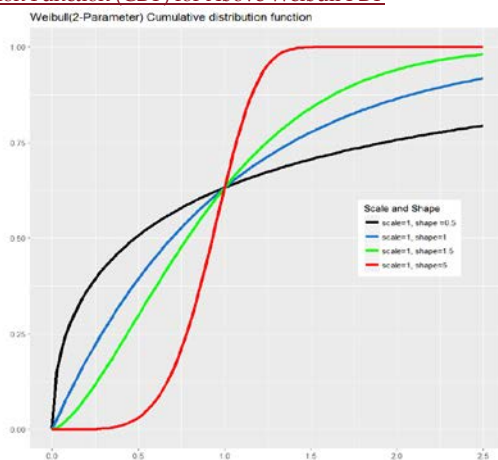


Figure 2. Cumulative Distribution Function (CDF) for Above Weibull PDF



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Figure 3: Histograms of CPT distributions for various CPTs and P5MRs (assume CPTs are Weibull distributions)

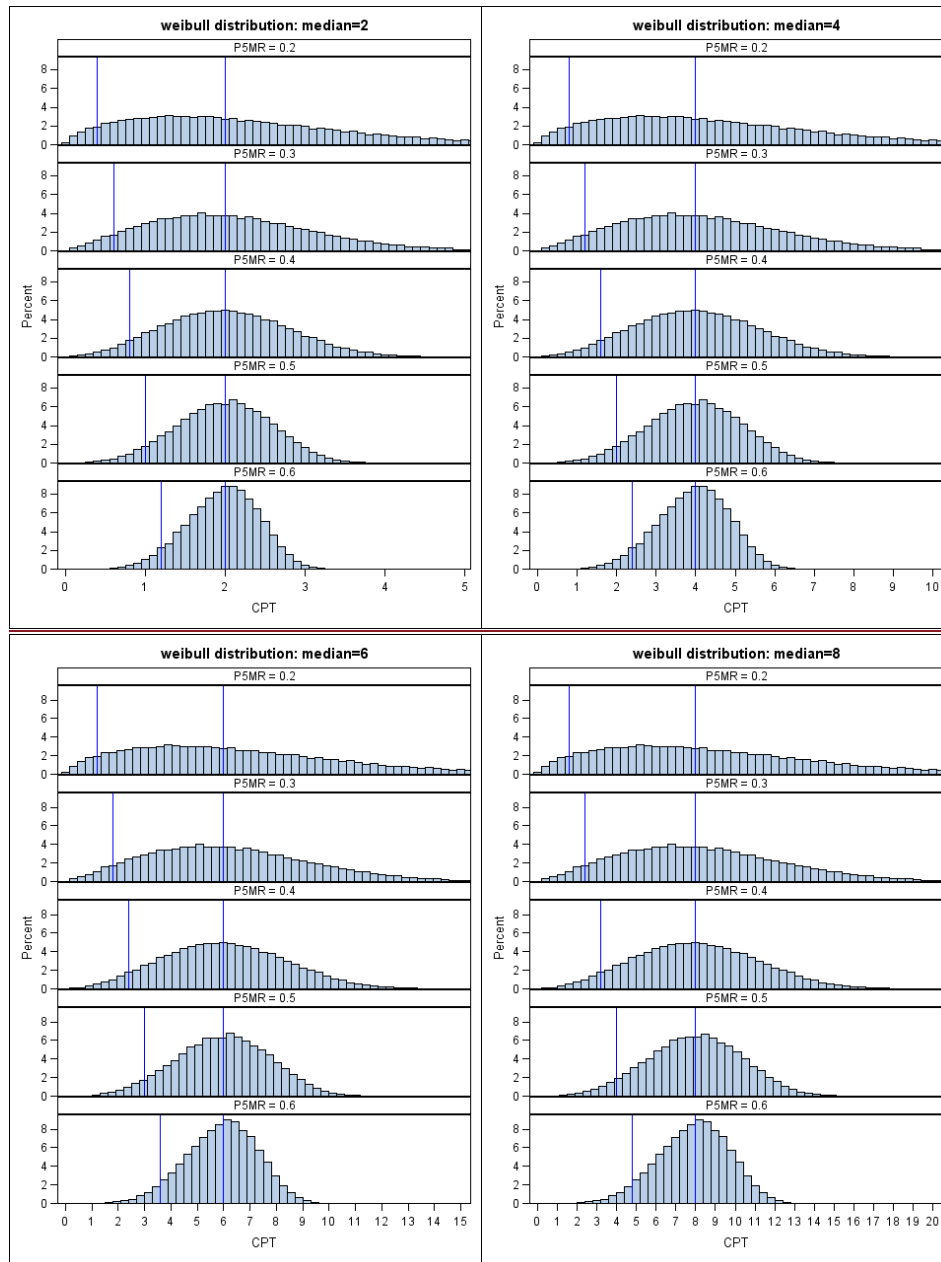


Table 1: Results of power analysis when the lowest acceptable ratio 95% LCL _{mCPT} /mCPT = 0.6 (Weibull distribution)												
Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.071	0.291	0.207	0.473	0.362	0.369	0.502	0.494	0.637	0.626	0.521
	0.4	0.297	0.691	0.594	0.841	0.804	0.779	0.893	0.898	0.939	0.945	0.932
	0.5	0.498	0.850	0.802	0.942	0.938	0.921	0.968	0.977	0.964	0.982	0.986
	0.6	0.733	0.949	0.943	0.962	0.971	0.955	0.954	0.979	0.915	0.951	0.971
	0.7	0.893	0.945	0.955	0.875	0.918	0.852	0.855	0.893	0.810	0.859	0.886
	0.8	0.819	0.786	0.826	0.666	0.734	0.591	0.637	0.708	0.558	0.632	0.689
4	0.2	0.043	0.208	0.146	0.356	0.289	0.254	0.432	0.380	0.567	0.516	0.435
	0.4	0.241	0.595	0.521	0.783	0.737	0.709	0.849	0.842	0.930	0.920	0.884
	0.5	0.412	0.795	0.730	0.921	0.901	0.888	0.956	0.964	0.986	0.988	0.973
	0.6	0.648	0.938	0.899	0.987	0.980	0.976	0.995	0.997	0.997	0.999	0.998
	0.7	0.869	0.988	0.986	0.993	0.995	0.994	0.992	0.998	0.977	0.992	0.996
	0.8	0.975	0.982	0.987	0.948	0.970	0.949	0.934	0.968	0.887	0.932	0.954
6	0.2	0.075	0.204	0.153	0.339	0.280	0.252	0.426	0.369	0.557	0.490	0.424
	0.4	0.227	0.572	0.504	0.759	0.743	0.689	0.851	0.826	0.929	0.916	0.885
	0.5	0.408	0.779	0.729	0.914	0.905	0.873	0.963	0.958	0.987	0.981	0.978
	0.6	0.645	0.925	0.906	0.984	0.980	0.977	0.997	0.997	1.000	0.999	0.999
	0.7	0.874	0.990	0.988	0.998	0.999	0.999	1.000	1.000	0.999	1.000	1.000
	0.8	0.986	0.998	0.999	0.993	0.995	0.994	0.990	0.997	0.975	0.989	0.995
8	0.2	0.323	0.346	0.362	0.457	0.443	0.361	0.537	0.453	0.636	0.564	0.522
	0.4	0.314	0.586	0.552	0.769	0.753	0.700	0.858	0.836	0.934	0.919	0.891
	0.5	0.421	0.779	0.732	0.914	0.904	0.875	0.960	0.956	0.989	0.985	0.979
	0.6	0.638	0.927	0.906	0.983	0.979	0.974	0.997	0.997	1.000	0.999	1.000
	0.7	0.874	0.990	0.989	0.999	1.000	0.999	1.000	1.000	1.000	1.000	1.000
	0.8	0.985	0.999	1.000	0.997	1.000	0.999	0.998	1.000	0.994	0.998	0.999

NOTE: Yellow indicates power > 0.8; orange indicates power > 0.9; blue indicates unusual power when median complete protection time = 2 hours and P5MR = 0.8.

Table 2: Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.7$ (Weibull distribution)												
Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.022	0.096	0.066	0.199	0.121	0.128	0.217	0.211	0.304	0.299	0.222
	0.4	0.132	0.415	0.299	0.607	0.484	0.522	0.634	0.677	0.751	0.767	0.668
	0.5	0.267	0.633	0.516	0.789	0.697	0.748	0.803	0.851	0.868	0.895	0.838
	0.6	0.476	0.813	0.732	0.881	0.845	0.864	0.885	0.919	0.893	0.926	0.918
	0.7	0.694	0.895	0.876	0.861	0.889	0.847	0.850	0.888	0.799	0.837	0.888
	0.8	0.768	0.780	0.821	0.673	0.750	0.591	0.652	0.699	0.566	0.622	0.694
4	0.2	0.016	0.075	0.053	0.166	0.109	0.088	0.190	0.171	0.276	0.245	0.177
	0.4	0.103	0.332	0.267	0.517	0.452	0.402	0.620	0.555	0.752	0.681	0.638
	0.5	0.210	0.525	0.468	0.715	0.685	0.624	0.830	0.776	0.914	0.866	0.848
	0.6	0.402	0.736	0.714	0.886	0.880	0.833	0.955	0.923	0.979	0.966	0.969
	0.7	0.673	0.914	0.917	0.971	0.975	0.962	0.987	0.986	0.982	0.988	0.995
	0.8	0.927	0.970	0.987	0.945	0.971	0.946	0.931	0.955	0.892	0.922	0.958
6	0.2	0.047	0.083	0.066	0.158	0.105	0.083	0.174	0.150	0.247	0.218	0.149
	0.4	0.079	0.294	0.225	0.473	0.387	0.356	0.556	0.507	0.690	0.636	0.566
	0.5	0.172	0.483	0.406	0.679	0.622	0.573	0.779	0.735	0.887	0.841	0.806
	0.6	0.335	0.697	0.649	0.861	0.851	0.804	0.938	0.909	0.977	0.963	0.956
	0.7	0.607	0.894	0.885	0.975	0.970	0.958	0.994	0.989	0.997	0.996	0.997
	0.8	0.897	0.987	0.992	0.988	0.995	0.993	0.989	0.993	0.978	0.987	0.995
8	0.2	0.309	0.234	0.297	0.289	0.320	0.210	0.347	0.251	0.392	0.306	0.306
	0.4	0.180	0.321	0.294	0.497	0.435	0.379	0.598	0.521	0.726	0.654	0.592
	0.5	0.206	0.499	0.439	0.692	0.645	0.603	0.804	0.762	0.904	0.867	0.830
	0.6	0.357	0.731	0.684	0.892	0.872	0.831	0.957	0.933	0.983	0.978	0.966
	0.7	0.634	0.922	0.907	0.985	0.981	0.976	0.998	0.994	0.998	0.998	0.999
	0.8	0.913	0.996	0.995	0.999	0.999	0.998	0.997	1.000	0.994	0.997	0.999

NOTE: Yellow indicates power > 0.8; orange indicates power > 0.9; blue indicates unusual power when median complete protection time = 2 hours and P5MR = 0.8.

Table 3: Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.8$ (Weibull distribution)												
Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20

Table 3: Results of power analysis when the lowest acceptable ratio 95% LCL _{mCPT} /mCPT = 0.8 (Weibull distribution)												
Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.007	0.032	0.027	0.064	0.044	0.027	0.079	0.061	0.115	0.088	0.071
	0.4	0.035	0.119	0.103	0.219	0.188	0.140	0.275	0.227	0.355	0.287	0.258
	0.5	0.068	0.210	0.185	0.339	0.297	0.251	0.418	0.364	0.506	0.460	0.425
	0.6	0.133	0.341	0.306	0.496	0.473	0.407	0.594	0.560	0.678	0.670	0.641
	0.7	0.251	0.561	0.532	0.679	0.692	0.598	0.753	0.753	0.749	0.775	0.809
	0.8	0.455	0.696	0.728	0.654	0.728	0.562	0.648	0.694	0.565	0.620	0.692
4	0.2	0.004	0.026	0.012	0.053	0.027	0.022	0.054	0.045	0.085	0.065	0.042
	0.4	0.026	0.093	0.080	0.186	0.151	0.103	0.254	0.182	0.346	0.245	0.228
	0.5	0.060	0.170	0.165	0.315	0.297	0.202	0.436	0.315	0.546	0.413	0.414
	0.6	0.135	0.317	0.320	0.494	0.499	0.374	0.650	0.529	0.760	0.643	0.639
	0.7	0.295	0.548	0.565	0.726	0.754	0.651	0.863	0.784	0.914	0.854	0.873
	0.8	0.619	0.828	0.867	0.884	0.923	0.864	0.913	0.922	0.886	0.909	0.947
6	0.2	0.038	0.033	0.027	0.055	0.037	0.027	0.053	0.033	0.076	0.058	0.039
	0.4	0.022	0.098	0.072	0.206	0.135	0.115	0.234	0.196	0.341	0.289	0.214
	0.5	0.054	0.205	0.154	0.364	0.281	0.248	0.438	0.382	0.567	0.493	0.425
	0.6	0.133	0.383	0.335	0.572	0.525	0.473	0.694	0.626	0.812	0.748	0.716
	0.7	0.316	0.646	0.614	0.819	0.818	0.750	0.918	0.874	0.965	0.943	0.938
	0.8	0.670	0.916	0.917	0.967	0.974	0.962	0.986	0.984	0.977	0.985	0.993
8	0.2	0.301	0.193	0.270	0.198	0.264	0.155	0.250	0.157	0.244	0.171	0.206
	0.4	0.122	0.136	0.141	0.227	0.182	0.142	0.267	0.208	0.340	0.292	0.229
	0.5	0.082	0.209	0.165	0.368	0.282	0.256	0.434	0.392	0.561	0.505	0.424
	0.6	0.124	0.390	0.321	0.588	0.514	0.490	0.688	0.655	0.823	0.779	0.710
	0.7	0.299	0.683	0.610	0.857	0.808	0.794	0.915	0.909	0.966	0.963	0.939
	0.8	0.644	0.940	0.909	0.989	0.978	0.981	0.994	0.995	0.993	0.995	0.998

NOTE: Yellow indicates power > 0.8; orange indicates power > 0.9.

Figure 4: Power curves of study design when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.6$ (Weibull distributions)

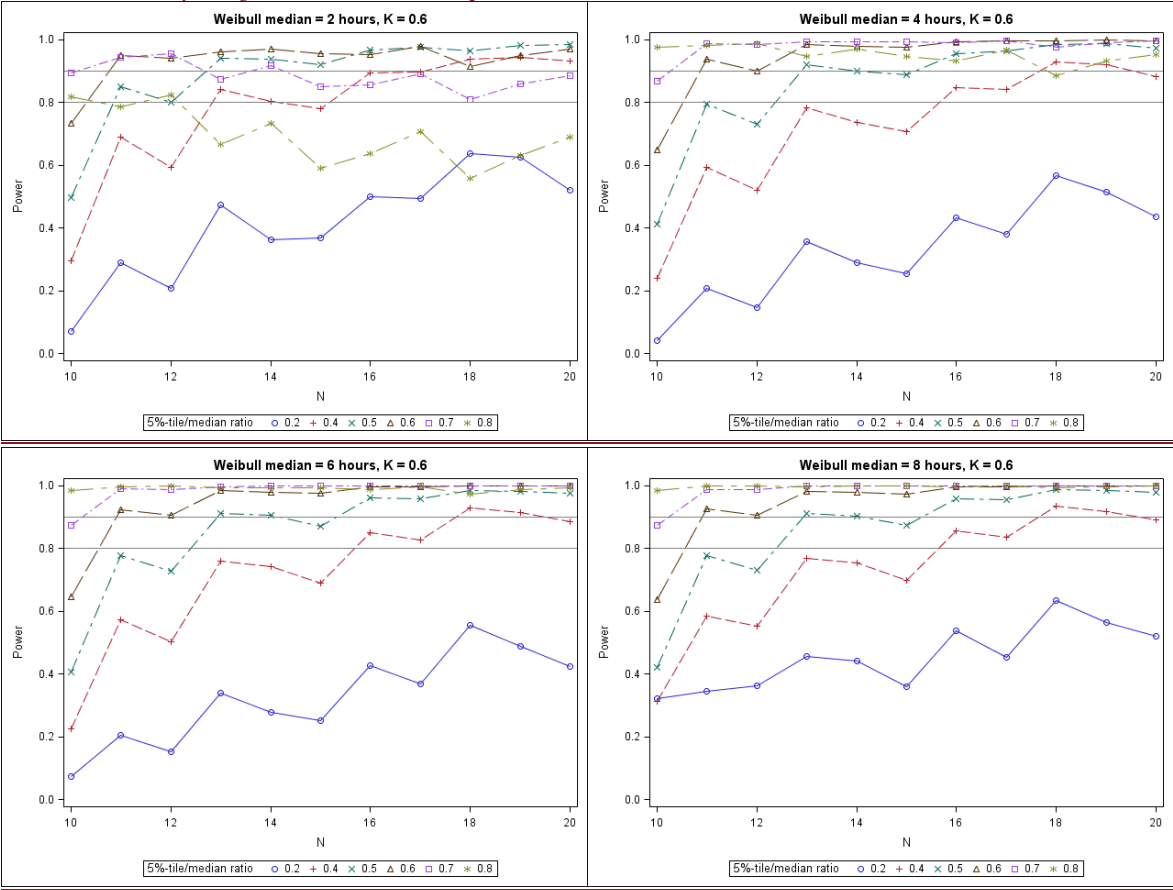


Figure 5: Power curves of study design when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.7$ (Weibull distributions)

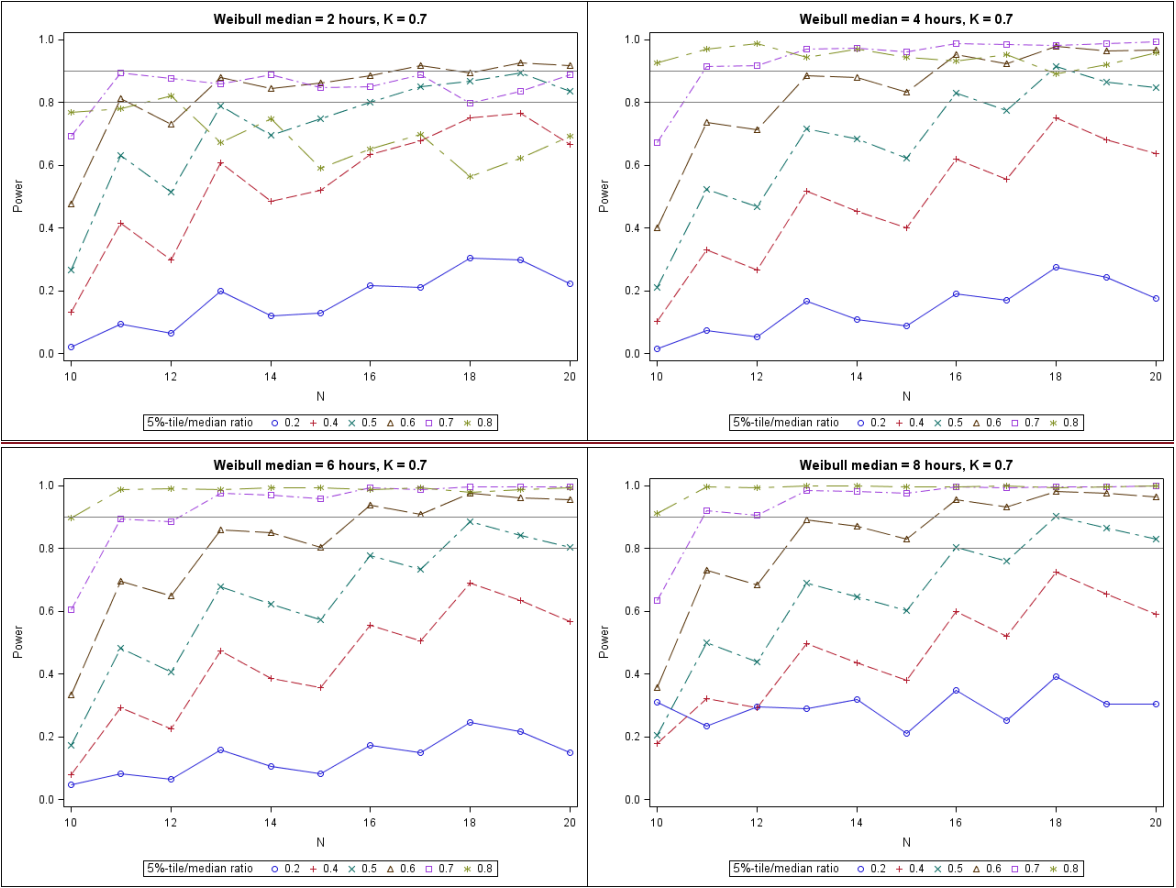
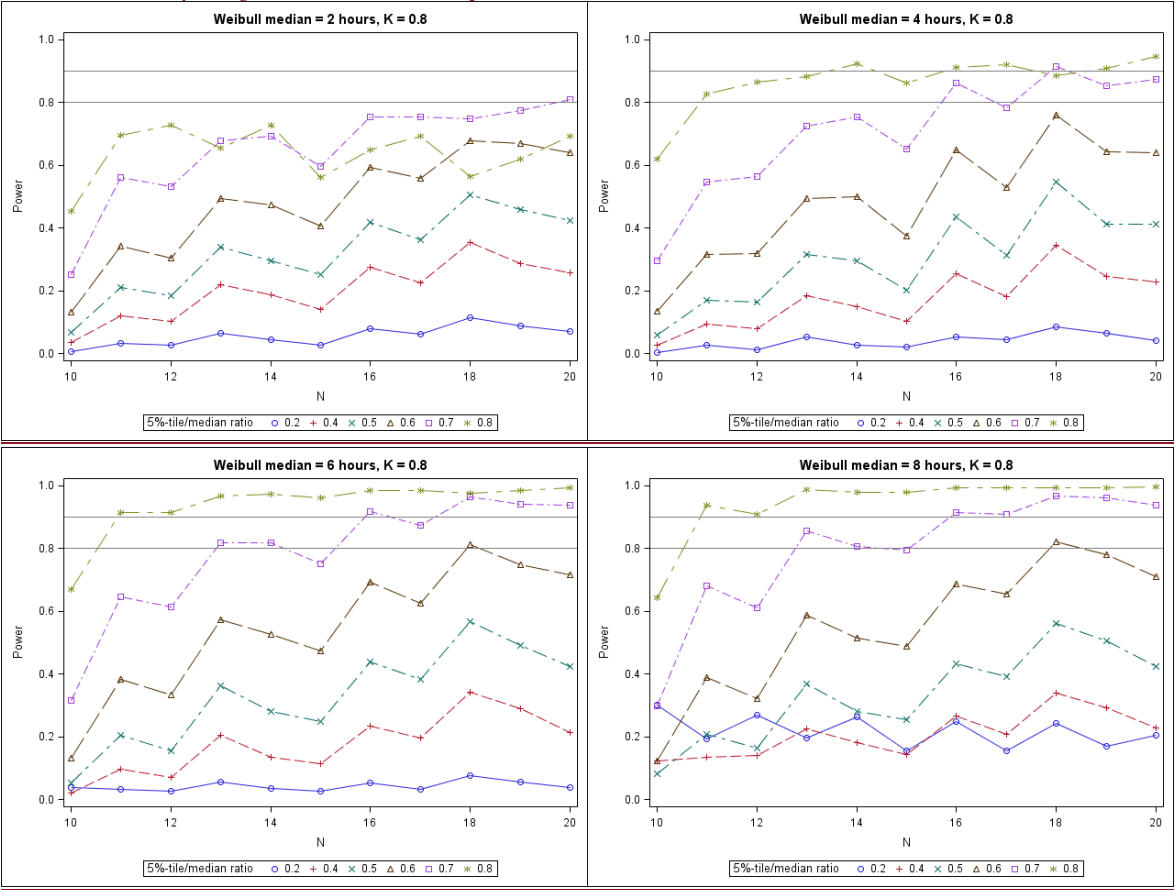


Figure 6: Power curves of study design when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.8$ (Weibull distributions)



APPENDIX 5A

Re-parameterization of Standard Weibull Equation

Given the definition of PDF and CDF from first principles:

$$P(mCPT, \kappa, \lambda) = 1 - e^{-\left(\frac{mCPT}{\lambda}\right)^\kappa} = 0.5 \quad (\text{median})$$

$$P(P5MR \times mCPT, \kappa, \lambda) = 1 - e^{-\left(\frac{P5MR \times mCPT}{\lambda}\right)^\kappa} = 0.05 \quad (\text{5th percentile})$$

Then:

$$e^{-\left(\frac{mCPT}{\lambda}\right)^\kappa} = 0.5 \quad (\text{median})$$

$$e^{-\left(\frac{P5MR \times mCPT}{\lambda}\right)^\kappa} = 0.05 \quad (\text{5th percentile})$$

and

$$-\left(\frac{mCPT}{\lambda}\right)^\kappa = \ln(0.5) \quad (1)$$

$$-\left(\frac{P5MR \times mCPT}{\lambda}\right)^\kappa = \ln(0.05) \quad (2)$$

Divide (2) by (1), we have:

$$\left[\frac{P5MR \times mCPT}{\lambda} \right]^\kappa = \frac{\ln(0.05)}{\ln(0.5)}$$

$$\kappa = \ln \left[\frac{\ln(0.05)}{\ln(0.5)} \right] / \ln(P5MR) \quad (3)$$

From (1):

$$\left(\frac{mCPT}{\lambda}\right)^\kappa = -\ln(0.5)$$

$$\kappa \times \ln \left(\frac{mCPT}{\lambda}\right) = \ln[-\ln(0.5)]$$

$$\ln \left(\frac{mCPT}{\lambda}\right) = \frac{1}{\kappa} \ln[-\ln(0.5)]$$

$$\ln(mCPT) - \ln(\lambda) = \frac{1}{\kappa} \ln[-\ln(0.5)]$$

$$\ln(\lambda) = \ln(mCPT) - \frac{1}{\kappa} \ln[-\ln(0.5)]$$

$$\begin{aligned}
 &= \frac{1}{\kappa} [\kappa \ln(mCPT) - \ln[-\ln(0.5)]] \\
 &= \frac{1}{\kappa} [\ln(mCPT^\kappa) - \ln[-\ln(0.5)]] \\
 &= \frac{1}{\kappa} \ln \left[-\frac{mCPT^\kappa}{\ln(0.5)} \right] \\
 \lambda &= e^{\frac{1}{\kappa} \ln \left[-\frac{mCPT^\kappa}{\ln(0.5)} \right]} \quad (4)
 \end{aligned}$$

So...

$$\begin{aligned}
 \kappa &= \ln \left[\frac{\ln(0.95)}{\ln(0.5)} \right] / \ln(P5MR) \\
 \lambda &= e^{\frac{1}{\kappa} \times \ln \left[-\frac{mCPT^\kappa}{\ln(0.5)} \right]} \\
 &\quad \text{(As shown in the main text)}
 \end{aligned}$$

APPENDIX 5B

Product	Location	Sample size	Est. mCPT (95% CI)	Ratio of 95% LCL/est. mCPT	Est. Weibull (shape κ ; scale λ)	Est. P5MR
A	<u>1</u>	<u>10</u>	<u>7.5 (4.0, 8.0)</u>	<u>0.53</u>	<u>6.602; 7.777</u>	<u>0.674</u>
	<u>2</u>	<u>10</u>	<u>8.5 (4.5, 10.0)</u>	<u>0.53</u>	<u>5.855; 8.624</u>	<u>0.641</u>
B	<u>1</u>	<u>10</u>	<u>12.0 (6.0, 12.0)</u>	<u>0.50</u>	<u>4.311; 10.669</u>	<u>0.547</u>
	<u>2</u>	<u>10</u>	<u>12.0 (8.5, 12.0)</u>	<u>0.71</u>	<u>10.424; 11.516</u>	<u>0.779</u>
C	<u>1</u>	<u>10</u>	<u>7.5 (4.0, 9.0)</u>	<u>0.53</u>	<u>4.430; 8.146</u>	<u>0.556</u>
	<u>2</u>	<u>8</u>	<u>5.0 (2.5, 5.5)</u>	<u>0.50</u>	<u>5.318; 4.915</u>	<u>0.613</u>
D	<u>1</u>	<u>10</u>	<u>2.0 (1.5, 2.0)</u>	<u>0.75</u>	<u>7.004; 2.135</u>	<u>0.690</u>
	<u>2</u>	<u>10</u>	<u>2.5 (1.0, 3.5)</u>	<u>0.40</u>	<u>3.557; 2.8970</u>	<u>0.481</u>
E	<u>1</u>	<u>10</u>	<u>8.25 (6.0, 10.0)</u>	<u>0.73</u>	<u>7.609; 8.733</u>	<u>0.710</u>
	<u>2</u>	<u>10</u>	<u>8.0 (3.5, 8.5)</u>	<u>0.44</u>	<u>4.009; 7.442</u>	<u>0.522</u>

APPENDIX 5C

SAS codes

```

=====
* Programmer: James Nguyen, USEPA
*
* Project: Mosquito Repellency Studies
*
* Purpose: Power Analysis/sample size calculation
*
* Description:
*   - distributions: Weibull, Normal, Lognormal, Uniform
*   - create histograms of the distributions
*   - SAS Procedures: PROC LIFETEST and PROC ICLIFETEST
*
* Review Date: 4/10/2017
=====
options formdlim=" " ps=90 ls=90 nonumber nodate;

libname MOS "C:\Users\JNguyen\Desktop\MOS";

%Macro distParam;
  if upcase(Distribution) = "WEIBULL" then do;
    * Weibull = f(x,a,b);
    a = log(log(0.95)/log(0.5))/log(P5MR);          b = exp((1/a)*log(-
(MED**a)/log(0.5)));
  end;
  if upcase(Distribution) = "UNIFORM" then do;
    * uniform = U[a, b];
    a = MED*(0.5*P5MR - 0.05)/0.45;          b = MED*2 - a;
  end;
  if upcase(Distribution) = "NORMAL" then do;
    * normal = N(a,b);
    a = MED;          b = MED*(1-P5MR)/1.645;
  end;
  if upcase(Distribution) = "LOGNORMAL" then do;
    * lognormal = exp(N(a,b));
    a = log(MED);          b = (log(MED)-log(MED*P5MR))/1.645;
  end;
%Mend;title;

%Macro generate;
  if upcase(Distribution) = "WEIBULL" then CPT = rand("Weibull", a, b);
  if upcase(Distribution) = "LOGNORMAL" then CPT = exp(rand("Normal", a, b));
  if upcase(Distribution) = "NORMAL" then CPT = rand("Normal", a, b);
  if upcase(Distribution) = "UNIFORM" then CPT = a + (b-a)*rand("Uniform");
%Mend;

%Macro Histogram(MED=, P5MRS=, dist=, seed=);

  %let N=1;
  %let P5MR&N = %nrquote(%scan(&P5MRS,&N, %str( )));
  %do %while (&&P5MR&N ^=);
    %let N=%eval(&N+1);
    %let P5MR&N = %nrquote(%scan(&P5MRS,&N, %str( )));
  %end;
  %let N=%eval(&N-1);

  Data Parameters;

```

```

MED = &MED;
%do i = 1 %to &N;
P5MR = &&P5MR&i;
P5 = MED*P5MR;
output;
%end;
label MED = "P50";
run;

Data Parameters;
set Parameters;
Distribution = "&dist";
%distParam;
run;

data simmer;
call streaminit(&seed);
set parameters;
do i = 1 to 50000;
%generate;
output;
end; *i;
drop i a b;
run;

title "&dist distribution: median=&MED";
Proc SGPanel data = Simmer;
panelby P5MR/rows=&N;
Histogram CPT/binwidth=%sysevalf(2.5*&MED/50);
refline P5 /axis=x lineattrs=(pattern=1 thickness=1 color=blue);
refline MED/axis=x lineattrs=(pattern=1 thickness=1 color=blue);
colaxis values = (0 to %sysevalf(2.5*&MED) by 1);
run;
Proc datasets nolist; save sasmacr; run;quit;
%Mend;title;

%Histogram(MED=2, P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);
%Histogram(MED=4, P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);
%Histogram(MED=6, P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);
%Histogram(MED=8, P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);
%Histogram(MED=10, P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);

%Macro Histogram1(MED=, P5MR=, seed=);

Data Parameters;
MED = &MED;
P5MR = &P5MR;
P5 = MED*P5MR;

do i = 1 to 4;
if i = 1 then Distribution = "Lognormal";
if i = 2 then Distribution = "Normal";
if i = 3 then Distribution = "Uniform";
if i = 4 then Distribution = "Weibull";
%distParam;
output;
end;
label MED = "P50" P5MR="5%-tile/median"; drop i;
run;

data simmer;
call streaminit(&seed);
set parameters;
do i = 1 to 50000;
%generate;
output;
end; *i;
drop i a b;

```

```

run;

title "median=&MED    P5MR=&P5MR" ;
Proc SGPANEL data = Simmer;
panelby Distribution/rows=4;
Histogram CPT/binwidth=%sysevalf(2.5*&MED/50);
refline P5 /axis=x lineattrs=(pattern=1 thickness=1 color=blue);
refline MED/axis=x lineattrs=(pattern=1 thickness=1 color=blue);
colaxis values = (0 to %sysevalf(2.5*&MED) by 1);

run;
Proc datasets nolist; save sasmacr; run;quit;
%Mend;title;

%Histogram1(MED=2, P5MR=0.2, seed=279420);
%Histogram1(MED=2, P5MR=0.4, seed=279420);
%Histogram1(MED=2, P5MR=0.5, seed=279420);
%Histogram1(MED=2, P5MR=0.6, seed=279420);
%Histogram1(MED=2, P5MR=0.7, seed=279420);

%Macro CPT;
CPT=CPT*60;
if CPT <= 5 then do;
LT = 0; RT = 0; CPT= 0; censor = 0;
end;
else if CPT >= &maxT*60 then do;
LT = &maxT*60; RT=.; CPT=&maxT*60; censor = 1;
end;
else do;
LT = 30*floor((CPT-5)/30)+5; RT = 30*ceil((CPT-5)/30); CPT = RT;
censor = 0;
end;

CPT = CPT/60;
LT = LT/60;
RT = RT/60;
%Mend;title;

%Macro power;
ods select none;
%if &censor=right %then %do;
ods output Quartiles=MPT;
Proc lifetest data = Simmer(keep=MED P5MR N Sim CPT Censor);
by MED P5MR N Sim;
time CPT*Censor(1);
run;
%end;
%if &censor=interval %then %do;
ods output quartiles=MPT;
Proc iclifetest data = simmer(keep=MED P5MR N Sim LT RT) method=turnbull
impute(seed=1234);
by MED P5MR N Sim;
time (LT, RT);
run;
%end;
ods select default;

Proc datasets nolist; delete simmer; run;quit;

Data MPT;
set MPT;
if percent = 50;
power = (LowerLimit >= &K*Estimate);
%if &censor=right %then %do; Censor = "right";%end;
%if &censor=interval %then %do; Censor = "interval"; %end;
run;

Proc SQL;
create table &dist&MED as

```

```

select Censor, MED, P5MR, N, avg(Power) as Power
from MPT
group by Censor, MED, P5MR, N;
quit;

%Mend;title;

%Macro Mosquito(med=, P5MRS=, nmin=,nmax=,maxT=,K=,dist=,censor=,NSim=, seed=);

    %let N=1;
    %let P5MR&N = %nrquote(%scan(&P5MRS,&N, %str( )));
    %do %while (&&P5MR&N ^=);
        %let N=%eval(&N+1);
        %let P5MR&N = %nrquote(%scan(&P5MRS,&N, %str( )));
    %end;
    %let N=%eval(&N-1);

    %do i = 1 %to &N;

        %if &i = 1 %then %do; data All_&dist&MED; set _NULL_; run; %end;

        Data Parameters;
            MED = &MED;
            P5MR = &&P5MR&i;
            P5 = MED*P5MR;
            label MED = "median" P5MR="5%-tile/median ratio";
        run;

        Data Parameters;
            set Parameters;
            Distribution = "&dist";
            %distParam;
        run;

        data simmer;
            call streaminit(&seed);
            set Parameters;
            do N = &Nmin to &Nmax;
                do Sim = 1 to &NSim;
                    do ID = 1 to N;
                        %generate;
                        output;
                    end; *ID;
                end; *Sim;
            end; *N;
            drop a b;
        run;

        Data Simmer;
            set Simmer;
            %CPT;
        run;

        %power;

        Data All_&dist&MED;
            set All_&dist&MED &dist&MED;
        run;

        Proc datasets nolist; delete Parameters simmer MPT &dist&MED; quit;

    %end;

    Data MOS_&dist._&censor._MED&MED._K%sysevalf(100*&K);
        set All_&dist&MED;
    run;

    Proc datasets nolist; save sasmacr; run;quit;

%Mend;

```

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```

dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Normal, censor=right, NSim=4000, seed=352);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Normal, censor=right, NSim=4000, seed=352);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Uniform, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Uniform, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Uniform, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Uniform, censor=right, NSim=4000, seed=561);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Uniform, censor=right, NSim=4000, seed=352);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Uniform, censor=right, NSim=4000, seed=352);

/*
dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Weibull, censor=interval, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Weibull, censor=interval, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Weibull, censor=interval, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Weibull, censor=interval, NSim=4000, seed=352);
*/

*====> Create Figures and Print Results;

libname MOS "C:\Users\JNguyen\Desktop\MOS";
%let folder=C:\Users\JNguyen\Desktop\MOS;

%Macro SGPLOT(distribution=, K=);
    title "&distribution median = 2 hours, K = 0.&K";
    Proc SGPlot data = MOS.&distribution._right_med2_k&k.0;
        scatter x = N y = Power/group = P5MR;

```

```

series x = N y = Power/group = P5MR;
refline 0.8 0.9/axis=y;
yaxis min=0 max=1;

run;
title "&distribution median = 4 hours, K = 0.&K";
Proc SGPlot data = MOS.&distribution._right_med4_k&k.0;
scatter x = N y = Power/group = P5MR;
series x = N y = Power/group = P5MR;
refline 0.8 0.9/axis=y;
yaxis min=0 max=1;

run;
title "&distribution median = 6 hours, K = 0.&K";
Proc SGPlot data = MOS.&distribution._right_med6_k&k.0;
scatter x = N y = Power/group = P5MR;
series x = N y = Power/group = P5MR;
refline 0.8 0.9/axis=y;
yaxis min=0 max=1;

run;
title "&distribution median = 8 hours, K = 0.&K";
Proc SGPlot data = MOS.&distribution._right_med8_k&k.0;
scatter x = N y = Power/group = P5MR;
series x = N y = Power/group = P5MR;
refline 0.8 0.9/axis=y;
yaxis min=0 max=1;

run;
%Mend;

%Macro print(distribution=, K=);
data &distribution._K&K;
set MOS.&distribution._right_med2_k&k.0
MOS.&distribution._right_med4_k&k.0
MOS.&distribution._right_med6_k&k.0
MOS.&distribution._right_med8_k&k.0;

run;
Proc transpose data = &distribution._K&K out = &distribution._K&K(drop=_NAME_);
by MED P5MR;
ID N;
var Power;

run;
title "&distribution K=0.&K.0";
Proc print data = &distribution._K&K noobs label; format _: 6.3; run;
%Mend;

%SGPLOT(distribution=Weibull, K=6);
%SGPLOT(distribution=Weibull, K=7);
%SGPLOT(distribution=Weibull, K=8);

%SGPLOT(distribution=Lognormal, K=6);
%SGPLOT(distribution=Lognormal, K=7);
%SGPLOT(distribution=Lognormal, K=8);

%SGPLOT(distribution=Normal, K=6);
%SGPLOT(distribution=Normal, K=7);
%SGPLOT(distribution=Normal, K=8);

%SGPLOT(distribution=Uniform, K=6);
%SGPLOT(distribution=Uniform, K=7);
%SGPLOT(distribution=Uniform, K=8);

ods rtf file = "&folder\&dist Median=&MED K=&K.rtf" bodytitle;
%print(distribution=Weibull, K=6);
%print(distribution=Weibull, K=7);
%print(distribution=Weibull, K=8);

%print(distribution=Lognormal, K=6);
%print(distribution=Lognormal, K=7);
%print(distribution=Lognormal, K=8);

```

```
%print(distribution=Normal, K=6);  
%print(distribution=Normal, K=7);  
%print(distribution=Normal, K=8);  
  
%print(distribution=Uniform, K=6);  
%print(distribution=Uniform, K=7);  
%print(distribution=Uniform, K=8);  
ods rtf close;
```

APPENDIX 5D

Table 4-1. Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.6$ (Lognormal distribution)												
Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.045	0.223	0.126	0.344	0.239	0.257	0.355	0.362	0.440	0.467	0.353
	0.4	0.236	0.580	0.476	0.776	0.666	0.678	0.815	0.812	0.900	0.903	0.869
	0.5	0.449	0.820	0.770	0.937	0.902	0.899	0.958	0.962	0.982	0.985	0.984
	0.6	0.768	0.969	0.955	0.979	0.988	0.980	0.980	0.991	0.963	0.981	0.988
	0.7	0.964	0.971	0.985	0.933	0.961	0.931	0.926	0.948	0.886	0.915	0.940
	0.8	0.894	0.867	0.914	0.801	0.851	0.761	0.797	0.839	0.743	0.792	0.827
4	0.2	0.048	0.169	0.103	0.259	0.188	0.162	0.295	0.254	0.381	0.343	0.279
	0.4	0.176	0.472	0.411	0.682	0.595	0.578	0.749	0.735	0.851	0.844	0.796
	0.5	0.367	0.729	0.662	0.895	0.834	0.828	0.924	0.927	0.973	0.972	0.960
	0.6	0.638	0.932	0.896	0.984	0.977	0.972	0.989	0.992	0.998	0.999	0.997
	0.7	0.919	0.996	0.991	0.999	0.999	0.998	0.997	0.999	0.995	0.998	0.999
	0.8	0.994	0.992	0.997	0.976	0.990	0.981	0.971	0.984	0.949	0.971	0.979
6	0.2	0.175	0.207	0.202	0.283	0.240	0.199	0.343	0.266	0.417	0.355	0.304
	0.4	0.180	0.474	0.400	0.677	0.600	0.561	0.751	0.706	0.845	0.827	0.794
	0.5	0.360	0.703	0.665	0.876	0.826	0.804	0.930	0.916	0.971	0.963	0.956
	0.6	0.635	0.917	0.900	0.982	0.976	0.964	0.992	0.993	0.999	0.998	0.999
	0.7	0.922	0.994	0.993	1.000	0.999	0.999	1.000	1.000	0.999	1.000	1.000
	0.8	0.999	0.999	1.000	0.998	0.999	0.998	0.996	0.999	0.993	0.998	0.999
8	0.2	0.408	0.389	0.438	0.449	0.470	0.371	0.535	0.418	0.594	0.501	0.487
	0.4	0.378	0.567	0.551	0.739	0.697	0.635	0.813	0.766	0.886	0.864	0.842
	0.5	0.469	0.742	0.731	0.898	0.868	0.831	0.942	0.924	0.979	0.967	0.963
	0.6	0.680	0.923	0.919	0.987	0.983	0.966	0.994	0.992	0.998	0.999	0.998
	0.7	0.929	0.994	0.994	1.000	1.000	0.999	1.000	1.000	1.000	1.000	1.000
	0.8	0.999	1.000	1.000	1.000	1.000	0.999	1.000	1.000	0.999	1.000	1.000

Table 4-2: Results of power analysis when the lowest acceptable ratio 95% LCL _{mCPT} /mCPT = 0.7 (Lognormal distribution)												
Median (hours)	P5M R	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.008	0.048	0.034	0.110	0.070	0.063	0.117	0.106	0.172	0.159	0.106
	0.4	0.072	0.285	0.197	0.465	0.355	0.390	0.489	0.530	0.608	0.619	0.519
	0.5	0.197	0.529	0.419	0.697	0.591	0.641	0.699	0.750	0.783	0.811	0.720
	0.6	0.440	0.749	0.648	0.856	0.775	0.816	0.844	0.893	0.877	0.907	0.874
	0.7	0.677	0.878	0.831	0.896	0.895	0.870	0.886	0.933	0.855	0.909	0.926
	0.8	0.800	0.866	0.905	0.796	0.849	0.744	0.795	0.843	0.737	0.786	0.837
4	0.2	0.024	0.056	0.037	0.110	0.064	0.058	0.107	0.093	0.160	0.139	0.090
	0.4	0.057	0.227	0.173	0.411	0.312	0.297	0.462	0.423	0.593	0.541	0.469
	0.5	0.157	0.430	0.368	0.627	0.561	0.521	0.714	0.669	0.819	0.765	0.745
	0.6	0.353	0.666	0.643	0.838	0.827	0.769	0.909	0.890	0.958	0.928	0.935
	0.7	0.692	0.894	0.906	0.966	0.970	0.945	0.988	0.983	0.992	0.993	0.996
	0.8	0.954	0.986	0.993	0.977	0.991	0.979	0.970	0.985	0.945	0.965	0.982
6	0.2	0.161	0.128	0.142	0.153	0.149	0.099	0.165	0.118	0.200	0.163	0.121
	0.4	0.068	0.210	0.157	0.368	0.279	0.258	0.402	0.361	0.542	0.487	0.403
	0.5	0.127	0.379	0.300	0.591	0.487	0.466	0.646	0.614	0.775	0.727	0.669
	0.6	0.284	0.623	0.559	0.809	0.773	0.735	0.880	0.867	0.941	0.912	0.914
	0.7	0.608	0.879	0.873	0.964	0.959	0.944	0.988	0.981	0.997	0.995	0.997
	0.8	0.941	0.993	0.996	0.996	0.999	0.998	0.998	0.999	0.995	0.997	0.999
8	0.2	0.394	0.311	0.381	0.331	0.382	0.274	0.390	0.296	0.407	0.320	0.333
	0.4	0.273	0.317	0.326	0.461	0.415	0.331	0.525	0.432	0.628	0.540	0.500
	0.5	0.260	0.439	0.406	0.621	0.577	0.504	0.712	0.650	0.824	0.771	0.731
	0.6	0.362	0.663	0.617	0.844	0.822	0.775	0.904	0.891	0.959	0.941	0.933
	0.7	0.653	0.905	0.898	0.979	0.975	0.972	0.993	0.992	0.999	0.999	0.998
	0.8	0.959	0.999	0.998	0.999	1.000	1.000	1.000	1.000	0.999	1.000	1.000

Table 4-3 Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.8$ (Lognormal distribution)												
Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.005	0.022	0.020	0.043	0.031	0.019	0.050	0.032	0.074	0.044	0.033
	0.4	0.021	0.067	0.062	0.144	0.117	0.087	0.178	0.134	0.252	0.193	0.164
	0.5	0.043	0.142	0.125	0.253	0.212	0.157	0.288	0.246	0.378	0.334	0.283
	0.6	0.094	0.253	0.219	0.414	0.364	0.301	0.487	0.448	0.571	0.549	0.510
	0.7	0.210	0.491	0.465	0.661	0.649	0.571	0.734	0.750	0.771	0.797	0.809
	0.8	0.534	0.794	0.824	0.780	0.833	0.723	0.792	0.839	0.736	0.785	0.836
4	0.2	0.019	0.022	0.014	0.042	0.025	0.021	0.029	0.028	0.048	0.046	0.019
	0.4	0.014	0.073	0.044	0.146	0.101	0.075	0.155	0.119	0.228	0.173	0.140
	0.5	0.036	0.129	0.109	0.237	0.207	0.151	0.311	0.226	0.412	0.300	0.303
	0.6	0.095	0.253	0.256	0.399	0.404	0.297	0.540	0.409	0.654	0.513	0.532
	0.7	0.258	0.462	0.496	0.649	0.666	0.558	0.782	0.688	0.861	0.770	0.801
	0.8	0.623	0.779	0.834	0.880	0.914	0.845	0.926	0.914	0.923	0.916	0.951
6	0.2	0.157	0.098	0.119	0.088	0.108	0.061	0.095	0.054	0.096	0.066	0.061
	0.4	0.028	0.068	0.046	0.138	0.092	0.073	0.144	0.125	0.203	0.184	0.121
	0.5	0.033	0.141	0.094	0.266	0.187	0.176	0.292	0.257	0.408	0.355	0.285
	0.6	0.078	0.292	0.228	0.469	0.397	0.358	0.541	0.482	0.680	0.606	0.560
	0.7	0.253	0.552	0.513	0.737	0.721	0.655	0.839	0.799	0.917	0.862	0.877
	0.8	0.680	0.882	0.904	0.962	0.971	0.946	0.989	0.982	0.992	0.992	0.996
8	0.2	0.392	0.279	0.366	0.270	0.348	0.244	0.332	0.245	0.315	0.234	0.289
	0.4	0.240	0.194	0.230	0.235	0.245	0.169	0.267	0.193	0.305	0.237	0.214
	0.5	0.173	0.203	0.193	0.301	0.266	0.195	0.337	0.275	0.435	0.368	0.306
	0.6	0.145	0.301	0.251	0.495	0.414	0.369	0.538	0.516	0.680	0.639	0.558
	0.7	0.250	0.588	0.502	0.773	0.714	0.707	0.837	0.841	0.916	0.888	0.876
	0.8	0.660	0.910	0.901	0.978	0.973	0.971	0.993	0.990	0.998	0.998	0.997

Table 4-4: Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.6$ (Normal distribution)												
Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.139	0.454	0.358	0.655	0.556	0.540	0.722	0.701	0.824	0.820	0.770
	0.4	0.326	0.711	0.653	0.877	0.825	0.818	0.916	0.912	0.960	0.962	0.952
	0.5	0.506	0.858	0.833	0.946	0.934	0.922	0.966	0.974	0.967	0.981	0.985
	0.6	0.730	0.951	0.949	0.963	0.978	0.962	0.958	0.979	0.934	0.957	0.969
	0.7	0.925	0.957	0.976	0.908	0.940	0.901	0.900	0.923	0.842	0.882	0.915
	0.8	0.875	0.846	0.899	0.768	0.821	0.723	0.760	0.812	0.695	0.753	0.791
4	0.2	0.098	0.360	0.291	0.569	0.476	0.448	0.646	0.618	0.775	0.757	0.692
	0.4	0.253	0.635	0.562	0.838	0.764	0.749	0.880	0.874	0.950	0.949	0.926
	0.5	0.415	0.800	0.747	0.938	0.901	0.899	0.965	0.960	0.989	0.990	0.982
	0.6	0.638	0.936	0.913	0.991	0.983	0.975	0.992	0.994	0.996	0.998	0.999
	0.7	0.888	0.993	0.987	0.996	0.998	0.995	0.994	0.998	0.988	0.996	0.997
	0.8	0.991	0.988	0.995	0.967	0.983	0.973	0.959	0.980	0.934	0.958	0.970
6	0.2	0.088	0.344	0.272	0.552	0.461	0.426	0.648	0.594	0.777	0.743	0.693
	0.4	0.246	0.607	0.558	0.820	0.761	0.719	0.889	0.864	0.956	0.936	0.932
	0.5	0.408	0.780	0.745	0.930	0.905	0.876	0.966	0.955	0.990	0.986	0.984
	0.6	0.638	0.927	0.918	0.987	0.982	0.973	0.996	0.995	1.000	0.998	1.000
	0.7	0.893	0.993	0.992	1.000	0.999	0.998	1.000	1.000	0.999	1.000	1.000
	0.8	0.997	0.999	0.999	0.996	0.998	0.997	0.994	0.998	0.988	0.996	0.997
8	0.2	0.231	0.395	0.362	0.574	0.504	0.459	0.667	0.605	0.780	0.756	0.705
	0.4	0.303	0.618	0.578	0.821	0.765	0.723	0.891	0.860	0.959	0.941	0.934
	0.5	0.422	0.785	0.759	0.933	0.910	0.879	0.967	0.956	0.991	0.986	0.987
	0.6	0.646	0.927	0.920	0.989	0.984	0.972	0.997	0.994	1.000	0.999	0.999
	0.7	0.896	0.992	0.991	1.000	1.000	0.998	1.000	1.000	1.000	1.000	1.000
	0.8	0.997	1.000	1.000	1.000	1.000	0.999	0.999	1.000	0.998	0.999	1.000

**Table 4-5: Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.7$
(Normal distribution)**

Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.044	0.216	0.143	0.390	0.287	0.293	0.429	0.441	0.566	0.559	0.450
	0.4	0.136	0.465	0.347	0.662	0.549	0.592	0.684	0.727	0.790	0.813	0.728
	0.5	0.262	0.627	0.521	0.803	0.710	0.744	0.801	0.857	0.857	0.892	0.840
	0.6	0.460	0.796	0.712	0.891	0.836	0.854	0.877	0.925	0.883	0.920	0.916
	0.7	0.690	0.885	0.860	0.882	0.895	0.855	0.867	0.915	0.822	0.879	0.908
	0.8	0.795	0.843	0.886	0.761	0.819	0.706	0.759	0.811	0.689	0.743	0.801
4	0.2	0.033	0.175	0.130	0.340	0.257	0.216	0.405	0.346	0.535	0.469	0.413
	0.4	0.111	0.369	0.317	0.581	0.516	0.466	0.683	0.622	0.811	0.746	0.709
	0.5	0.214	0.526	0.475	0.742	0.704	0.643	0.833	0.797	0.915	0.871	0.870
	0.6	0.391	0.718	0.697	0.882	0.881	0.821	0.944	0.925	0.976	0.957	0.964
	0.7	0.683	0.905	0.915	0.971	0.979	0.957	0.990	0.988	0.990	0.993	0.996
	0.8	0.940	0.983	0.993	0.966	0.984	0.968	0.961	0.978	0.926	0.957	0.973
6	0.2	0.028	0.153	0.105	0.300	0.215	0.191	0.345	0.301	0.483	0.426	0.347
	0.4	0.085	0.328	0.259	0.550	0.455	0.416	0.619	0.580	0.754	0.708	0.646
	0.5	0.174	0.486	0.416	0.710	0.648	0.600	0.794	0.764	0.886	0.851	0.827
	0.6	0.334	0.681	0.635	0.863	0.837	0.798	0.927	0.914	0.970	0.951	0.952
	0.7	0.607	0.892	0.885	0.971	0.969	0.958	0.991	0.989	0.997	0.996	0.998
	0.8	0.925	0.991	0.995	0.994	0.999	0.996	0.997	0.998	0.992	0.996	0.998
8	0.2	0.185	0.222	0.214	0.351	0.296	0.231	0.416	0.335	0.522	0.448	0.390
	0.4	0.162	0.350	0.308	0.560	0.492	0.428	0.653	0.603	0.784	0.730	0.680
	0.5	0.205	0.494	0.439	0.726	0.678	0.621	0.809	0.785	0.909	0.875	0.851
	0.6	0.358	0.713	0.662	0.890	0.870	0.835	0.945	0.932	0.981	0.971	0.970
	0.7	0.642	0.916	0.902	0.985	0.980	0.980	0.996	0.996	0.999	0.999	0.998
	0.8	0.943	0.998	0.998	0.999	1.000	0.999	1.000	1.000	0.998	0.999	1.000

Table 4-6: Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.8$ (Normal distribution)												
Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.013	0.069	0.049	0.142	0.109	0.081	0.176	0.136	0.250	0.193	0.154
	0.4	0.032	0.142	0.120	0.259	0.214	0.167	0.311	0.259	0.401	0.352	0.305
	0.5	0.067	0.210	0.183	0.355	0.314	0.245	0.426	0.382	0.504	0.469	0.433
	0.6	0.116	0.328	0.291	0.502	0.464	0.387	0.578	0.559	0.661	0.650	0.628
	0.7	0.239	0.537	0.508	0.683	0.685	0.601	0.741	0.776	0.760	0.799	0.824
	0.8	0.512	0.768	0.801	0.745	0.802	0.685	0.756	0.807	0.689	0.741	0.800
4	0.2	0.008	0.051	0.035	0.112	0.084	0.055	0.140	0.098	0.208	0.138	0.123
	0.4	0.029	0.114	0.093	0.224	0.192	0.133	0.304	0.214	0.420	0.293	0.295
	0.5	0.055	0.182	0.169	0.320	0.305	0.221	0.451	0.323	0.572	0.432	0.443
	0.6	0.118	0.289	0.310	0.479	0.484	0.363	0.633	0.503	0.750	0.620	0.632
	0.7	0.271	0.512	0.528	0.695	0.720	0.616	0.827	0.744	0.892	0.824	0.853
	0.8	0.618	0.800	0.844	0.892	0.923	0.854	0.927	0.925	0.911	0.920	0.952
6	0.2	0.005	0.051	0.031	0.115	0.070	0.054	0.129	0.104	0.196	0.155	0.105
	0.4	0.022	0.120	0.088	0.247	0.179	0.159	0.297	0.247	0.410	0.347	0.287
	0.5	0.044	0.206	0.163	0.377	0.297	0.265	0.453	0.389	0.584	0.509	0.457
	0.6	0.111	0.357	0.307	0.558	0.502	0.444	0.664	0.600	0.799	0.721	0.691
	0.7	0.285	0.605	0.573	0.796	0.786	0.716	0.885	0.861	0.949	0.907	0.921
	0.8	0.680	0.898	0.913	0.968	0.977	0.955	0.990	0.986	0.990	0.993	0.996
8	0.2	0.164	0.131	0.143	0.164	0.163	0.101	0.190	0.135	0.245	0.177	0.146
	0.4	0.097	0.152	0.128	0.260	0.204	0.158	0.298	0.248	0.411	0.353	0.283
	0.5	0.082	0.213	0.171	0.389	0.303	0.265	0.440	0.401	0.589	0.536	0.445
	0.6	0.119	0.362	0.290	0.591	0.496	0.465	0.660	0.635	0.789	0.759	0.695
	0.7	0.273	0.638	0.564	0.827	0.773	0.760	0.887	0.891	0.945	0.933	0.918
	0.8	0.658	0.923	0.909	0.983	0.976	0.978	0.995	0.995	0.998	0.998	0.998

**Table 4-7: Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.6$
(Uniform distribution)**

Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.059	0.266	0.177	0.434	0.319	0.331	0.471	0.450	0.576	0.586	0.491
	0.4	0.242	0.561	0.468	0.727	0.657	0.664	0.792	0.772	0.876	0.870	0.824
	0.5	0.429	0.788	0.736	0.923	0.880	0.889	0.955	0.949	0.981	0.982	0.974
	0.6	0.987	0.995	0.994	0.995	0.995	0.993	0.990	0.998	0.981	0.990	0.996
	0.7	0.996	0.990	0.994	0.970	0.981	0.968	0.960	0.977	0.931	0.961	0.975
	0.8	0.900	0.904	0.934	0.851	0.886	0.793	0.849	0.875	0.813	0.851	0.883
4	0.2	0.038	0.183	0.126	0.336	0.253	0.226	0.385	0.340	0.499	0.467	0.397
	0.4	0.175	0.446	0.372	0.641	0.577	0.547	0.723	0.675	0.814	0.807	0.743
	0.5	0.357	0.681	0.627	0.851	0.796	0.802	0.906	0.883	0.947	0.948	0.917
	0.6	0.693	0.919	0.876	0.975	0.956	0.960	0.986	0.984	0.996	0.996	0.991
	0.7	1.000	1.000	1.000	1.000	1.000	0.999	0.999	1.000	0.999	0.999	1.000
	0.8	0.999	0.997	1.000	0.996	0.996	0.994	0.990	0.998	0.981	0.990	0.996
6	0.2	0.037	0.161	0.114	0.313	0.228	0.198	0.368	0.312	0.485	0.442	0.384
	0.4	0.159	0.437	0.359	0.626	0.559	0.541	0.722	0.660	0.819	0.784	0.740
	0.5	0.371	0.653	0.620	0.823	0.797	0.766	0.895	0.853	0.946	0.930	0.910
	0.6	0.674	0.899	0.879	0.971	0.960	0.951	0.987	0.985	0.996	0.996	0.992
	0.7	0.970	0.998	0.999	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	1.000	1.000	1.000	1.000	1.000	0.999	0.999	1.000	0.999	0.999	1.000
8	0.2	0.291	0.302	0.323	0.423	0.390	0.299	0.499	0.375	0.564	0.491	0.458
	0.4	0.314	0.493	0.463	0.665	0.625	0.561	0.747	0.668	0.834	0.796	0.751
	0.5	0.452	0.687	0.668	0.835	0.813	0.784	0.905	0.872	0.949	0.934	0.918
	0.6	0.744	0.903	0.902	0.968	0.965	0.952	0.991	0.983	0.995	0.995	0.992
	0.7	0.977	0.998	0.997	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

**Table 4-8: Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.7$
(Uniform distribution)**

Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.016	0.079	0.052	0.168	0.117	0.098	0.184	0.186	0.277	0.267	0.197
	0.4	0.073	0.242	0.172	0.417	0.310	0.335	0.445	0.489	0.570	0.601	0.491
	0.5	0.180	0.470	0.371	0.645	0.540	0.584	0.662	0.711	0.725	0.780	0.683
	0.6	0.543	0.731	0.636	0.803	0.711	0.767	0.783	0.839	0.826	0.874	0.808
	0.7	0.624	0.847	0.757	0.890	0.853	0.855	0.885	0.914	0.896	0.931	0.918
	0.8	0.820	0.904	0.914	0.845	0.885	0.803	0.841	0.882	0.813	0.856	0.882
4	0.2	0.014	0.066	0.041	0.145	0.100	0.073	0.162	0.145	0.266	0.220	0.155
	0.4	0.055	0.203	0.148	0.351	0.285	0.251	0.425	0.379	0.557	0.494	0.432
	0.5	0.136	0.365	0.305	0.551	0.499	0.442	0.648	0.594	0.746	0.690	0.655
	0.6	0.354	0.600	0.572	0.750	0.761	0.670	0.854	0.807	0.908	0.872	0.874
	0.7	0.782	0.850	0.876	0.934	0.947	0.896	0.973	0.954	0.989	0.976	0.980
	0.8	1.000	0.999	1.000	0.994	0.995	0.995	0.992	0.995	0.984	0.991	0.996
6	0.2	0.016	0.055	0.035	0.129	0.079	0.062	0.141	0.120	0.224	0.190	0.125
	0.4	0.045	0.180	0.118	0.313	0.244	0.209	0.367	0.334	0.493	0.450	0.371
	0.5	0.116	0.330	0.252	0.496	0.421	0.384	0.572	0.525	0.672	0.638	0.581
	0.6	0.280	0.549	0.493	0.713	0.683	0.616	0.799	0.764	0.875	0.848	0.828
	0.7	0.665	0.839	0.846	0.931	0.933	0.896	0.970	0.956	0.992	0.979	0.982
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	0.999	1.000	1.000
8	0.2	0.268	0.210	0.267	0.257	0.268	0.179	0.297	0.215	0.358	0.267	0.244
	0.4	0.203	0.248	0.240	0.367	0.331	0.245	0.434	0.367	0.542	0.467	0.400
	0.5	0.203	0.356	0.318	0.513	0.467	0.394	0.615	0.554	0.713	0.660	0.612
	0.6	0.338	0.576	0.550	0.741	0.722	0.661	0.838	0.806	0.902	0.877	0.854
	0.7	0.693	0.892	0.882	0.959	0.956	0.942	0.982	0.980	0.995	0.990	0.990
	0.8	0.996	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

**Table 4-9: Results of power analysis when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.8$
(Uniform distribution)**

Median (hours)	P5MR	Sample size										
		10	11	12	13	14	15	16	17	18	19	20
2	0.2	0.009	0.031	0.023	0.065	0.053	0.031	0.081	0.051	0.120	0.091	0.070
	0.4	0.027	0.070	0.057	0.144	0.113	0.084	0.178	0.128	0.253	0.190	0.162
	0.5	0.047	0.121	0.119	0.227	0.192	0.142	0.275	0.211	0.344	0.278	0.246
	0.6	0.131	0.205	0.200	0.313	0.283	0.221	0.375	0.322	0.476	0.409	0.373
	0.7	0.142	0.364	0.315	0.530	0.483	0.418	0.609	0.595	0.703	0.692	0.651
	0.8	0.641	0.864	0.871	0.837	0.879	0.795	0.841	0.880	0.813	0.856	0.882
4	0.2	0.003	0.017	0.009	0.048	0.024	0.019	0.050	0.038	0.081	0.063	0.033
	0.4	0.013	0.061	0.038	0.121	0.090	0.066	0.138	0.103	0.205	0.160	0.122
	0.5	0.031	0.112	0.080	0.195	0.159	0.114	0.249	0.185	0.342	0.251	0.231
	0.6	0.092	0.198	0.194	0.309	0.325	0.211	0.428	0.317	0.528	0.403	0.428
	0.7	0.303	0.374	0.415	0.523	0.564	0.428	0.673	0.562	0.755	0.654	0.675
	0.8	0.766	0.735	0.806	0.805	0.860	0.769	0.902	0.839	0.916	0.874	0.899
6	0.2	0.006	0.015	0.009	0.041	0.019	0.017	0.042	0.029	0.069	0.054	0.029
	0.4	0.010	0.049	0.028	0.110	0.069	0.050	0.120	0.101	0.189	0.160	0.106
	0.5	0.026	0.101	0.065	0.197	0.142	0.114	0.214	0.201	0.327	0.284	0.219
	0.6	0.074	0.238	0.164	0.368	0.298	0.271	0.421	0.382	0.541	0.480	0.426
	0.7	0.246	0.461	0.422	0.608	0.611	0.521	0.732	0.656	0.808	0.751	0.748
	0.8	0.773	0.824	0.860	0.913	0.939	0.880	0.966	0.940	0.986	0.970	0.978
8	0.2	0.261	0.178	0.247	0.181	0.220	0.141	0.215	0.139	0.214	0.146	0.159
	0.4	0.175	0.129	0.162	0.175	0.176	0.100	0.203	0.141	0.245	0.184	0.143
	0.5	0.134	0.144	0.135	0.214	0.185	0.128	0.253	0.206	0.338	0.282	0.223
	0.6	0.119	0.224	0.182	0.354	0.289	0.254	0.418	0.378	0.529	0.492	0.407
	0.7	0.223	0.485	0.419	0.652	0.594	0.564	0.730	0.701	0.803	0.794	0.748
	0.8	0.679	0.868	0.851	0.939	0.942	0.916	0.976	0.968	0.991	0.984	0.987

Figure 4-1: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Lognormal distributions)

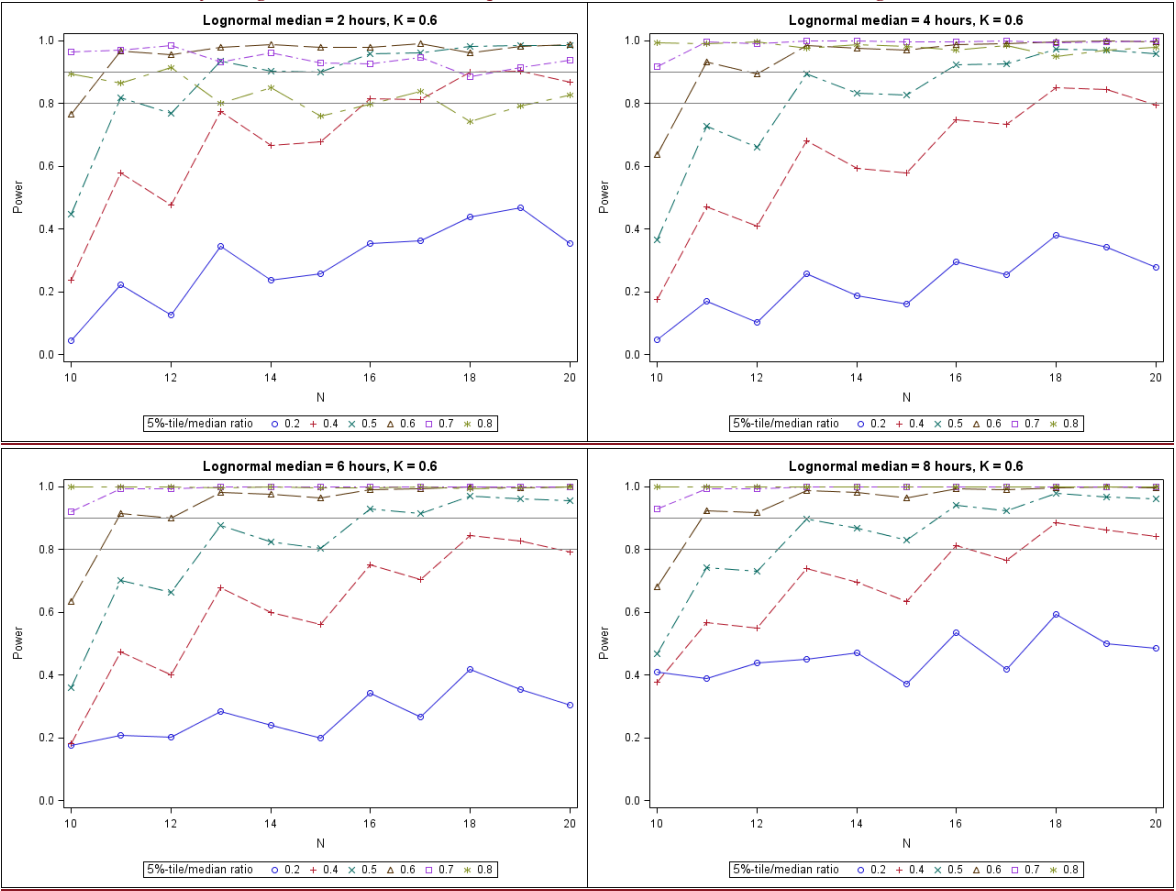


Figure 4-2: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Lognormal distributions)

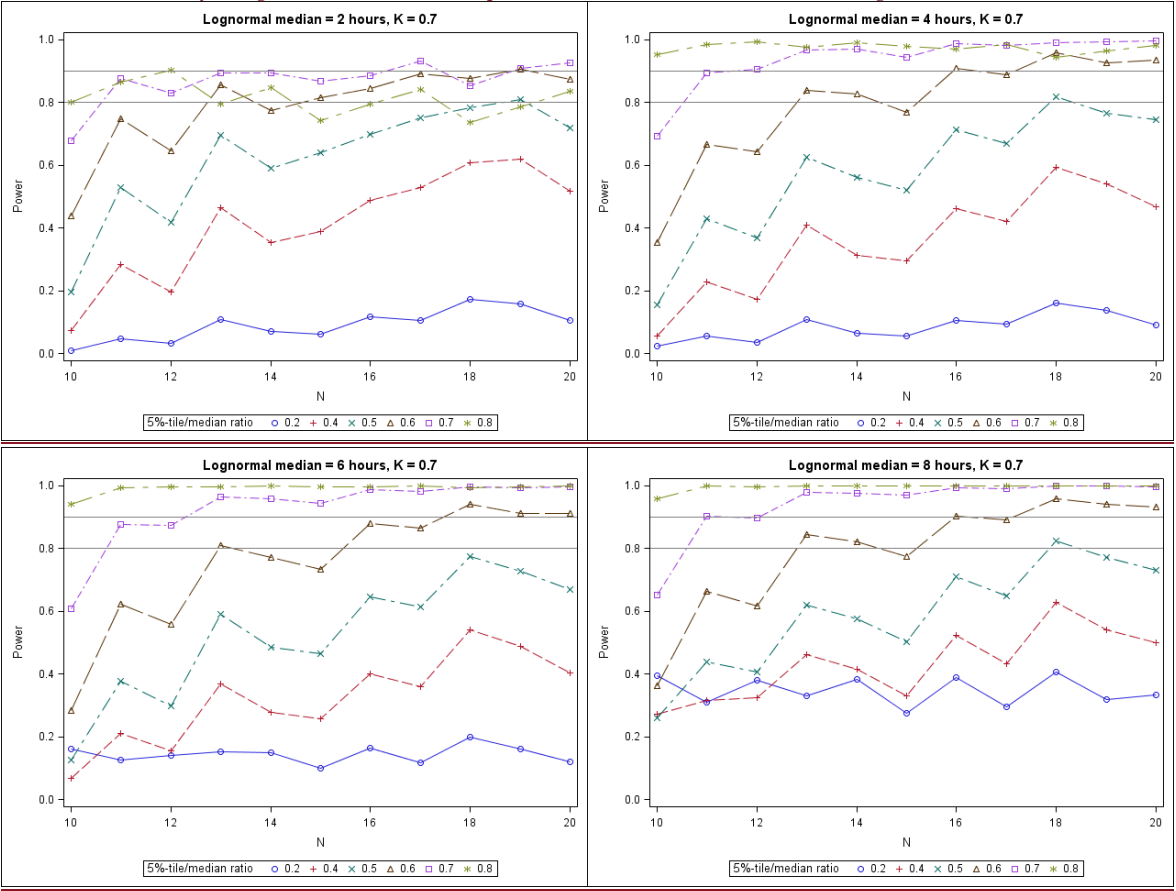


Figure 4-3: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Lognormal distributions)

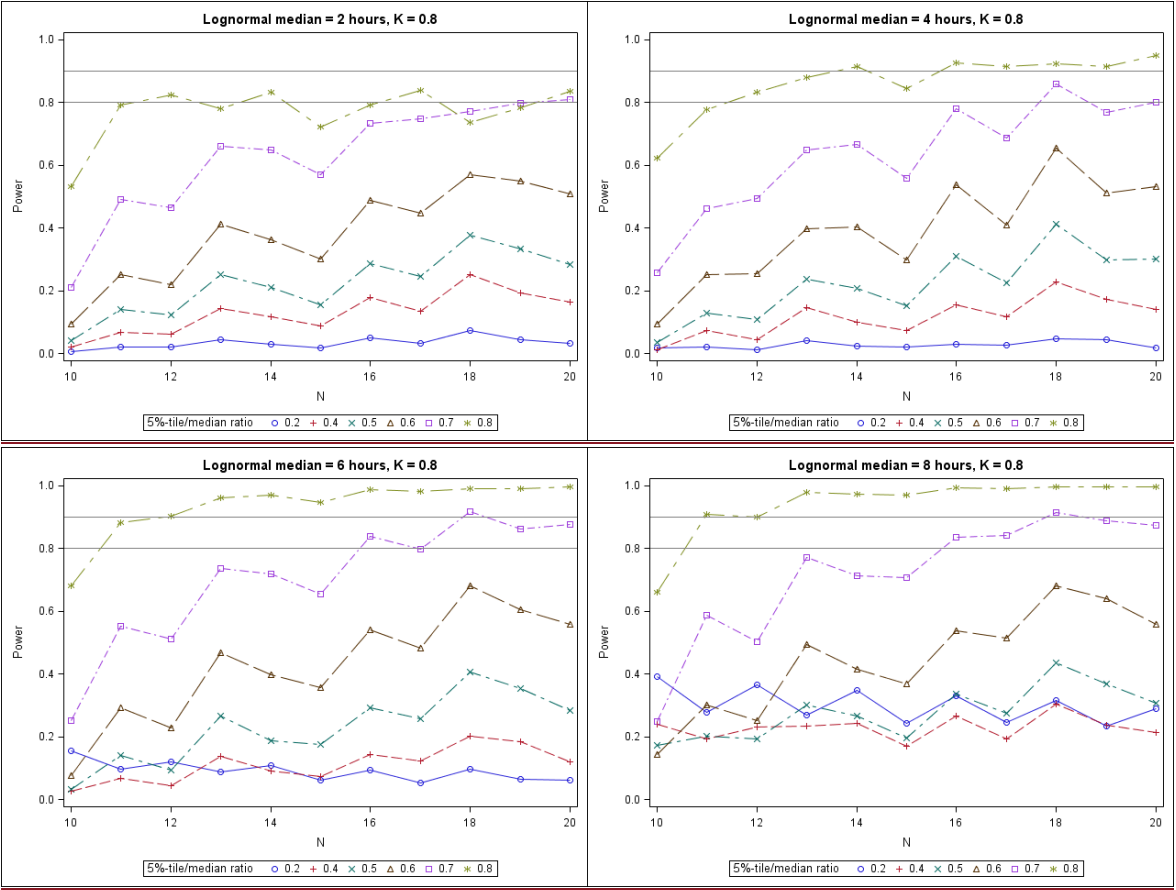


Figure 4-4: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Normal distributions)

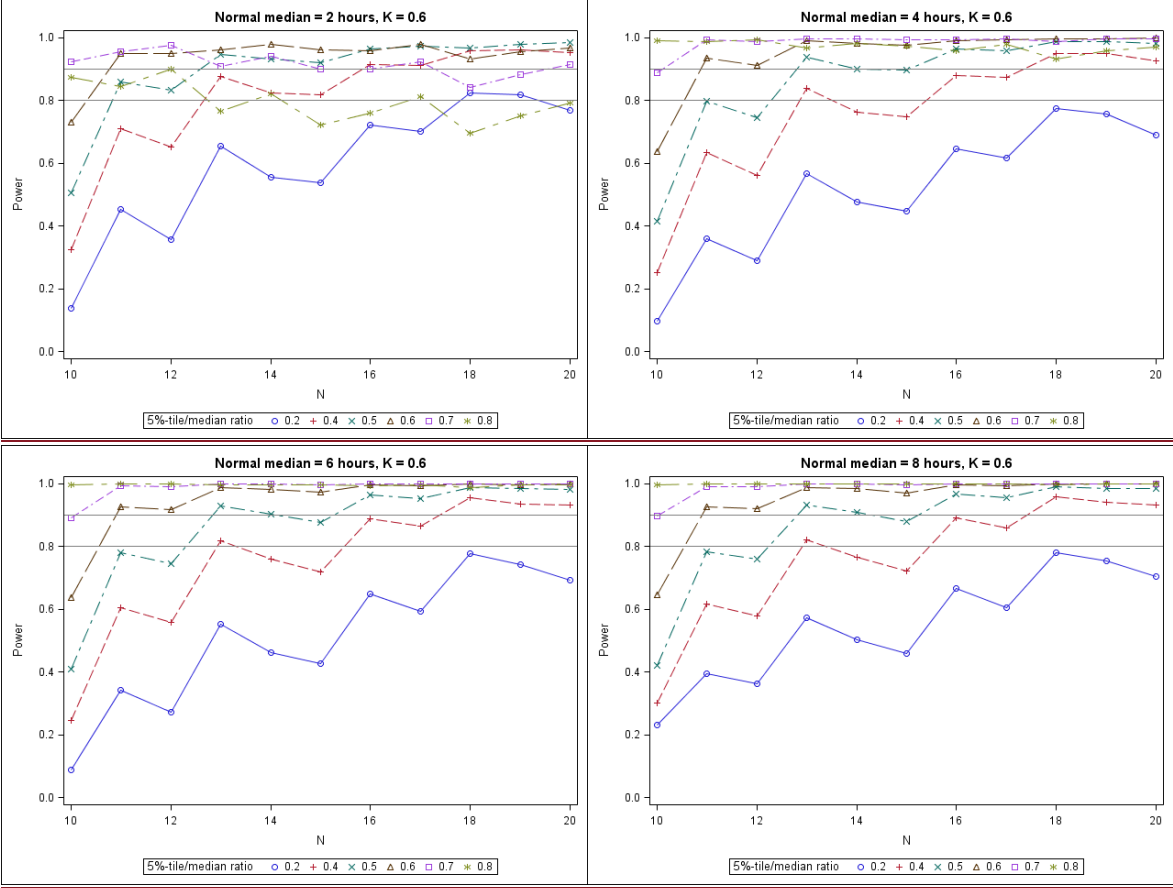


Figure 4-5: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Normal distributions)

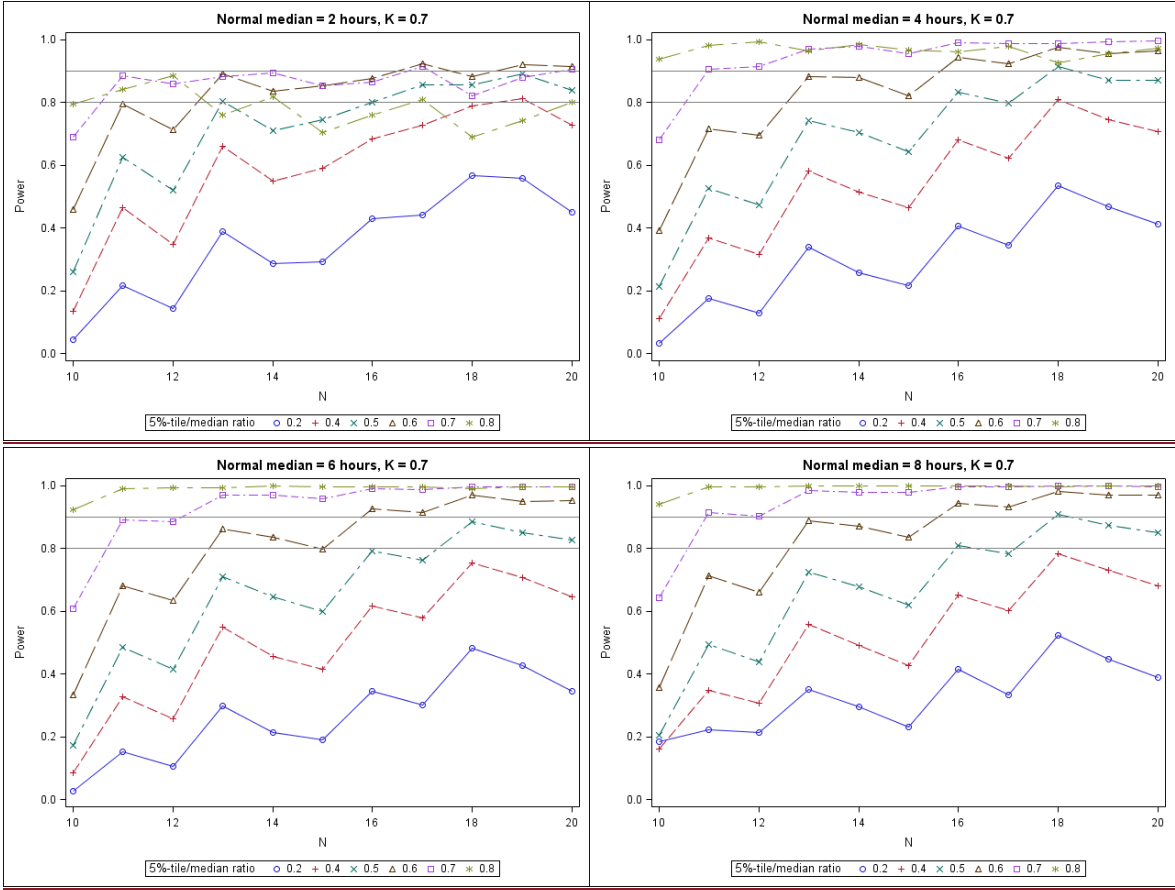


Figure 4-6: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Normal distributions)

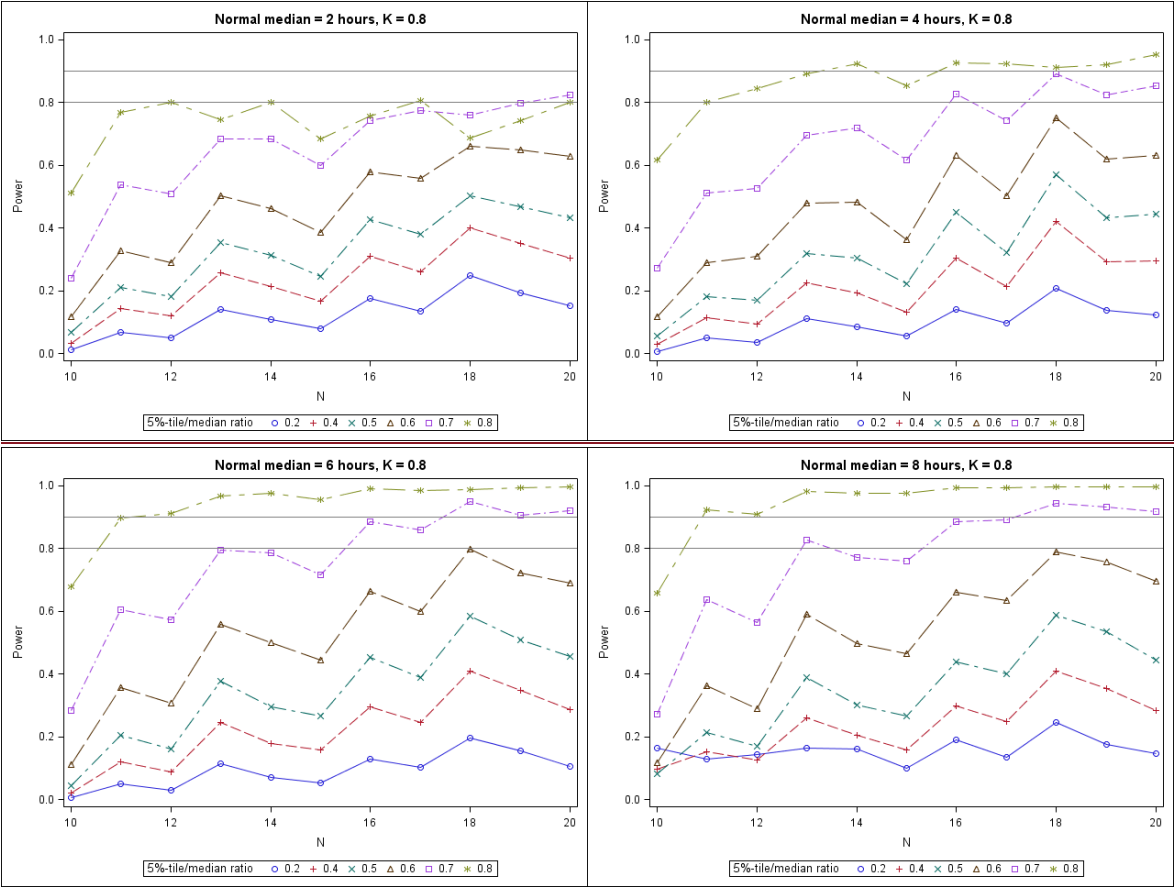
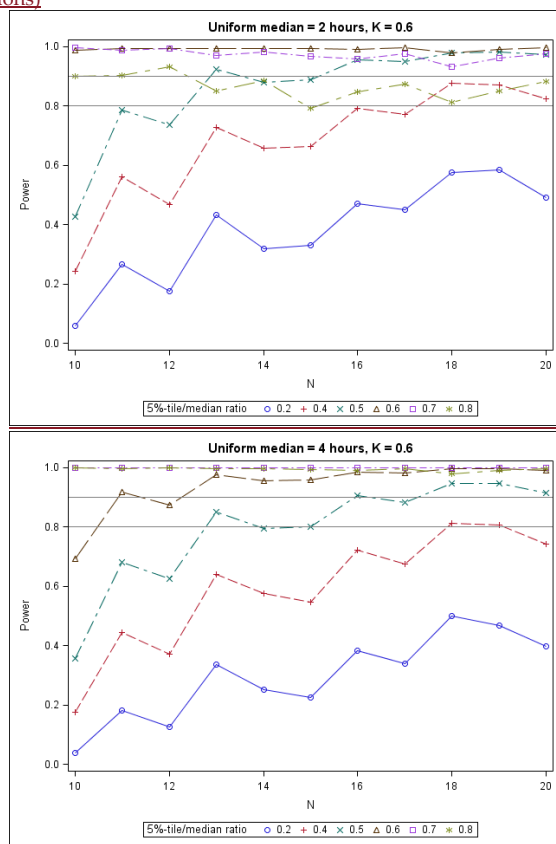


Figure 4-7: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Uniform distributions)



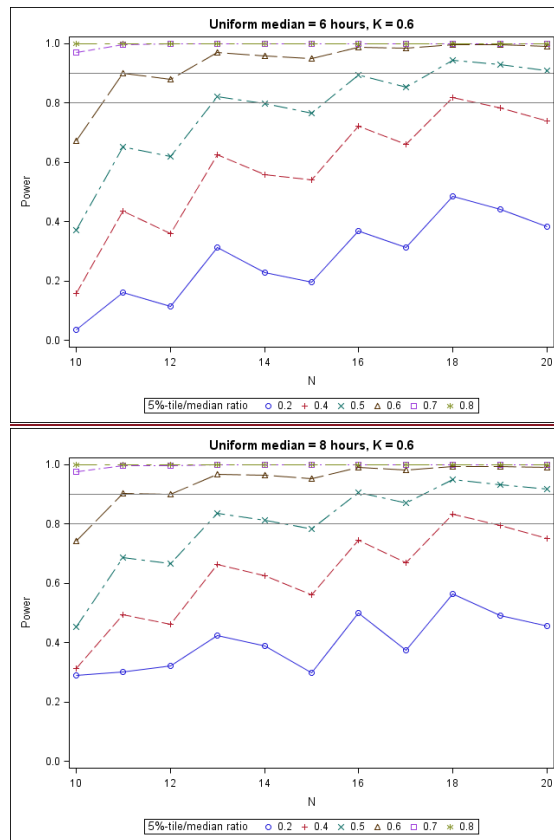
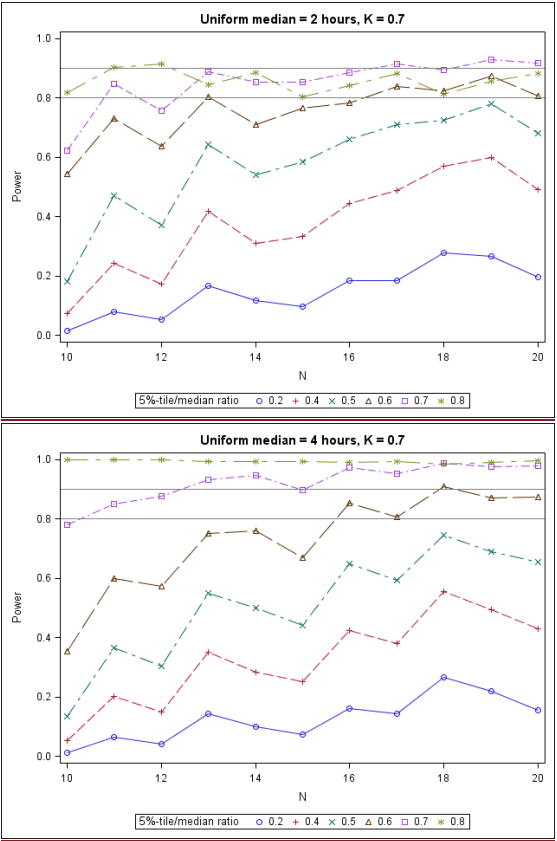


Figure 4-8: Power curves of study design when the lowest acceptable ratio 95% $LCL_{mCPT}/mCPT = 0.7$ (Uniform distributions)



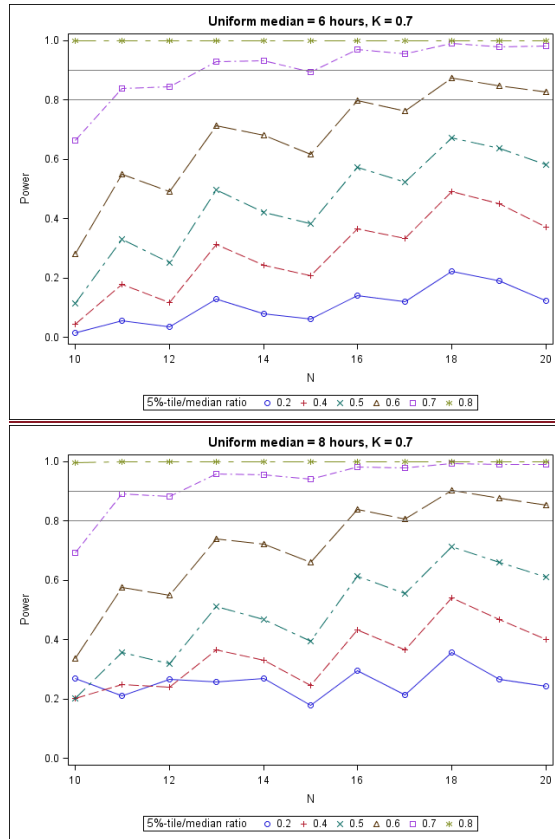


Figure 4-9: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Uniform distributions)

